

Advanced studies to use plant extracts against some insect pests

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The experiments performed in the laboratory of the plant protection Department, Faculty of Agriculture, Moshtohor, Zagazig university, Banha Branch. The aim of this work was to evaluate the activity of the botanical extracts from ten plants extracted by five solvents differ in their polarity against the cutworm, *Agrotis ipsilon* and rice weevil *Sitophilus oryzae*. For this purpose the following points were studied: 1-Evaluation the toxicity of selected plant extracts against adults of *Sitophilus oryzae* and *Agrotis ipsilon* larvae. 2- Effect of plant extracts on the development of the studied insect pests. 3-Evaluation of the antifeedant activity of these plant extracts against 4th instar larvae of *Agrotis*. 4-Histological effects of the most effective plant extracts against the mid gut of *Agrotis ipsilon*. 5-Evaluation of the repellent activity of plant extracts against the adults of *Sitophilus oryzae*. 6-Separation and identification of most effective compounds against both tested insects. 7-Determination the toxicity and biological activity of the identified compounds. The obtained results could be summarized in 4 the followings: Part 1- Effects of plant extracts on *Agrotis ipsilon*. *Results lead to the speculation that 10 % ethyl acetate extract of *Aptenia cordifolia* gave the highest value of antifeedant activity (93.87%) compared with other tested plants. *The results indicated that petroleum ether and ethyl alcohol extracts of *Thevetia neriifolia* at 10% concentration gave the highest number of *Agrotis ipsilon* dead larvae (19 & 18 respectively out of twenty tested). While ethyl acetate extract was the lowest effective one producing only 8 / 20 dead larvae. The pupation were inhibited totally by the petroleum ether extract at 10 % concentration. *Testing the effect of various plant extracts as stomach poisons confirmed that petroleum ether was the best solvent in extracting the most effective components. The extracts of *Glycine hispida* plant were the highest effective ones among the all used plants. Precisely 10 % concentration of all tested solvents gave not less than 90 % mortality. *The acetone and petroleum ether extracts of *Glycine hispida* were the most effective extracts against the larvae of *A. ipsilon* followed by ethyl alcohol and acetone extracts of *Thuja* seeds. *Data showed that the detachment of the muscle layer from the epithelium cells after treatment with extract of *Thuja orientalis*, *Glycine hispida* and *Aptenia cordifolia* lead to the lost of eating ability of treated larvae. *The damage of the epithelial cells resulted in stopping digestion and absorption of digested food, that resulted reduction in larval weight and development in comparison with control. *The extracts of *lupinus termis* or *cassia fistula* resulted in completely in disappearance of the fat bodies from the midgut cells. All spongy regenerative cells were destroyed in both treatments. Part 2- Effects of plant extracts on *Sitophilus oryzae*. *All plant extracts under investigation were examined for their stomach toxicity to the adults of *S. oryzae*. Results, showed that the most mortality percentages were proportional to the concentration and the time of exposure, in all experiments. *Seeds extracts of *Glycine hispida* on the adults of *S. oryzae* were the most effective between all plant extracts. Data showed 100 % initial kill only after the treatment with ethyl alcohol extracts at 10 % concentrations and 99.32 % mortality at 5 % concentration of the same extract. *The LC50 values were determined to evaluate the toxicity of the different extracts. Ethyl acetate extracts of *Glycine hispida* and *Cassia fistula* and ethyl alcohol extracts of *Glycine hispida* and *Schinus terebinthifolius* represent the highest toxicity among the tested extract. *The sap. fraction of *Glycine* extract caused 100 % initial mortality after the treatment with 1000 ppm concentration. The mortality was decreased by lowering the concentration but

increased with the elongation of exposure period. Although sap. or unsap. fractions of *A. codifolia* were moderately toxic under the same condition. *The bioassay results of separated compounds by TLC showed that six bands from ten were more effective than the four other bands. The most effective bands recorded 94.07 % and 89.15 % mortality against the larvae of *A. ipsilon* and the adults of *S. oryzae*. *The content of all isolated bands of TLC were effective compared to the control against the larvae of *Culex pipiens*. *The petroleum ether extract of *Schinus terebinthifolius* was the highest repellent one giving 100% after 3 days of exposure at a 10 % concentration and followed by the leaves and the seeds extracts of *Thuja orientalis* (93.18 % and 89.13 % after 3 days). *The essential oils extracted from *Schinus* leaves were more repellent than those extracted from *Thuja* leaves. *The essential oils Cedrol, β pinene, α phellandrene, α pinene exhibited biological activity in the form of repellency against *S. oryzae* adult more than that in control.

Part 3- Chemical investigation: *The chemical investigation illustrates that *Thevetia nervifolia*, *Cassia fistula* and *Aptenia cordifolia* were rich in carbohydrate. Although, *Glycine hispida* was rich in flavonoides, saponins and sterols. While, *Thuja orientalis* and *Schinus terebinthifolius* were rich in sterols or triterpenes. Finally, *Lupinus termis* seeds were rich in alkaloids or nitrogenous bases. *The most bioactive extracts were subjected to chemical analysis in order to separate and identify the active compounds in the four most effective extracts. *The chemical analysis of the essential oils of *Thuja orientalis* and *Schinus terebinthifolius* were made by using Gas Chromatography Mass Spectrophotometer (GC/MS). *The essential oils of *Thuja orientalis* revealed the presence of 65 peaks. The peaks were identified as [α —cedrol, linalool, β —carophyllene, germacrene — d, germacrene — b, camphene, α — phellandrene, limonene, β — phellandrene, α — elemene, (+)-2- carene, valencene, β — pinene, sabinene and T- muurolol]. While the GC/MS analysis of essential oil of *Schinus terebinthifolius* revealed the presence of 64 peaks. Only 15 components were identified as δ — cadinene, [3 — elemene, 1-phellandrene, 6 — elemene, [3 — pinene, δ 3- carene, para — cymene chlorofonn, δ — cadinene, δ — gurjunene, sabinene, α — pinene, (z) bis — 1,2 tri-methyl — silyloxy ethylene, 6 — terpinene and a terpinene. *Saponification of petroleum ether extract yielded saponifiable and unsaponifiable portions. The sap portion analysed by GLC. the Myristic acid was the predominant unsaturated Fatty acid in the composition of *Glycine hispida* with a percentage of 60.691 % followed by stearic acid with concentration of 24.258 %. Other Fatty acids were identified namely caproic acid, palmitoleic acid. Traces of other acids like linoleic acid, caproic acid and caprylic were determined. * Ethyl acetate extract of *A. cordifolia* was fractionated by preparative thin layer chromatography TLC. Ten spots were separated and appeared in these TLC plates.

Part 4-Side effect of the extracted essential oil: *The essential oils of *Thuja* were more reductive to germination of wheat grains than that extracted from *Schinus* such adverse effect was increased with the elongation of storage period after treatment. * α pinene was the material caused the highest reduction in germination, 71.12 % at 500 ppm compared with the control (6.79%) beginning. After the storage the treated grains recorded a reduction in germination (65.56%) of concentration of 500 ppm, compared with 2.23% in control. On the other hand, (+)—cedrol showed the lowest effect on the grain germination, it recorded a reduction of 34.34 % at 500 ppm. *The chlorophyll content decreased by increasing the concentration of essential oils in treatments. Although it increased with the prolongation of exposure period before germination of grains. *Essential oils decreased the amount of chlorophyll at the initial time such effect was is proportional to the concentration applied. *Effect of *Thuja* and *Schinus* essential oils on albino rats: -A tested concentration of 250 ppm was only extract leading to increase the number of erythrocytes, while the other two concentrations caused decreasing effect. *All treatments caused significant reduction in hemoglobin concentration. The essential oils caused more vacuolation of the cells and hemorrhage between cells with 250 ppm of *Thuja* extract. A photomicrographs of a section in treated rat liver with *Thuja* essential oils showed disturbed liver cord with inflammatory cells and vacuolation of the liver cells. *Schinus* product caused dilated central vein, increase of inflammatory cells vacuulations and hemorrhagic central vein. The same two plant products caused thickened basement membrane of the Bowman's capsules, infiltrations, inflammatory cells and decrease in the size of glomerulus's.