ENGLISH SUMMARY

Azotobacter Chroococcum, *Azotobacter vinelandii* and *Escherichia coli.* were subjected to genetic transformation in order to aquiresaponin for producing ability. The transforming materials in case of Azotobacter sp. were *Saponaria* high molecular weight DNA, *Saponaria officinalis* fresh roots DNA, *Poinciana regia* chromatin DNA and *Asparagus officinalis* fresh roots DNA, each of concentration 22 µg/ml. In case of *E.coli*, the transforming material was *Saponaria* fresh roots DNA of concentrations 66, 50 and 30 µg/ml.

Some biolgocial activites of saponin in each of liquid culture of transformant and untransformant *Azotobcter*, culture supernatant, cell suspensions and extracted (TLC) saponin bands of culture supernatants of isolate (1) of transformant, untransformant and isolate "2" of transformant *E. coli* were tested with comparison to aqueous extracts of 3 plants (*Saponaria*, *Poinciana* and *Asparagus*) of different concentrations as follow:

I. Haemolytic activity:

About 0.5 ml from each saponin solution mentioned above was added to 5 ml of blood corpuscles suspension, Kept at room temp. for 5 minutes, centrifuged at 3000 rpm for 15 minutes. The content of H.b in supernatant was measured in coloremeter at 540 O.D. The saponin content of unknown samples were calculated from the linear portion

of the standrd curve which was constructed for white saponin of Merck.

II Moluscicidal activity:

Ten snails (*Biomphalaria alexandrina)* were transferred to about 10 ml from each tested saponin solution and dechlorinated H20 as control for 24 hrs in duplicate then transferred to dechlorinated water for 24 hr. After recovery, mortality was calculated.

III. Nematicidal activity:

About 1000 2-nd stage juveniles were transferred to 10 ml of each tested saponin soultion and distilled water as control in triplicates for 24 hrs then were transferred to aerated water for 24 hrs. Mortality percentages of nematodes were calculated after recovery.

- Type of saponin in plants was determined by the colour produced by application of the liebermann- Burchard reaction.
- Various transformation frequencies in case of *Azotobacter* were observed. The highest frequency was obtained from the two types of *Saponaria* DNA.
- Transformant *E.Coli* were obtained with the concentration 66 µg/ml and the transformation frequency was 0.00096.
- Untransformant bacterial strain don't produce saponin and don't

exhibit any of its examined biological activities.

- Transformant strains exhibited various degrees of biological activites of saponin as follows:-

- The *Azotobacter chroococcum* transformed by *Saponaria* high molecular wieght DNA gave the highest (60%) molluscicidal activity. This mortality value was equal to that obtained from standard saponin (Merck) conc. of 0.015mg/ml). While the highest molluscicidal activity of cell suspensions of *E.coli* was that of the isolate (2) of transformed strain (30%, equivalent to 0.014 mg/ml of Merck saponin) which cause decrease in DNA content of snails to the lowest value (5.2 mg/g of snail tissue) with comparison to control which have (23.1 mg/g of snail tissue).

- The purified saponin extracted from culture supernatant of transformant *E.coli* mortalized snails at the maximum percentage (100%), which was equivalent to the mortality caused by 0.0175 mg/ml of Merck saponin, the DNA content of dead snail tissue was reduced to about 4.1 mg/g of snail tissue. Cell suspension and the spots dervied from the wild *E.Coli* showed no molluscicidal activity.

- The best potent saponin examined in 3 plants was that of *Saponaria* and *Asparagus* (LC_{50} were 35, 40 mg/ml) respectively for snails, which equivalent ot the mortality caused by 0.015 mg/ml of Merck saponin.

Nematicidal activity:

- *A. chroococcum* transformed by *Saponaria* high molecular weight DNA gave the highest nematodes mortality (50%) which corresponds to 80 mg/ml concentation of Merck saponin.

-Cells suspension of isolate (1) of transformant *E.Coli* gave the highest nematodes mortality percentage (60%) in comparison to the cell suspension of wild type and isolate (2) of transformant *E.Coli*

-Purified saponin extracted from culture supernatant of isolate (1) of transformant and isolate (2) of transformant *E.coli*. caused 100% nematodes mortality, equivalent to 160 mg/ml of Merck saponin.

- The best potent saponin examined in 3 plants was that of *Saponaria* and *Asparagus* LC_{50} for nematodes were 100 mg plant/ ml from each plant.

Haemolytic activity:

Culture of *A. chroococcum* transformed by fresh *Saponaria* DNA could hemolyze red blood cells to an extend equal to the obtained by 40.8 mg/ ml of standard saponin.

- Haemolytic activity of isolate(1) of transformant and isolate (2) of transformant *E.coli* cell suspensions was greater than that of the supernatants. Isolate (1) of transformed strain gave stronger hemolytic action than isolate (2) of transformed strain in both cases.

- The hemolytic activity of purified saponin extracted from culture supernatant of isolate (1) of transformant *E.coli* was lower than that of isolate (2) of transformant one (0.5 and 0.736) respectively.

- Aqueous extracts of *Saponaria* roots and *Poinciana* seeds at conc. of 100 mg plant/ ml gave stronger hemolytic action than aqueous extract of the same conc. from *Asparagus*, this may be due to the differene in saponin type (triterpenoid in *Saponaria* and *Poinciana*, but it its steroid in *Asparagus*).

- Aqueous extract of *Saponaria* could hemolyze red blood cells to an extend equal to that obtained by 0.015 mg/ml of standard saponin, so *Saponaria* is considered to be the most producer for saponin than *Asparagus* and *Poinciana*.