Potentiation of bacillus thuriniensis effectivenss against soodoptera littoralis through nontoxic agents

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5.1. Effect of kosmotropic and chaotropicmaterials on B.t Cry protein activity:The kosmotropic materials used were sucrose, glycerol and ethylene glycol. The tested concentrations were 5, 10, 15, 20 and 40 g (ml)/l. Data indicated that sucrose and glycerol kosmotropic materials increased the activity of B.t. Cry protein (Dipel-2X) at 2.5 g/1 . Sucrose (20g/1) increased mortality by 73% more than efficacy of Dipel alone after 2days post treatment, while glycerol achieved 67% increase by 40m1/1 after the same period. By elongation of feeding period to 4 and 7 days , sucrose (40g/1) increased activity of B.t Cry protein by 32% and 13% respectively, while the corresponding increase by glycerol (20m1/1) reached 27% and 10% respectively. All concentrations of ethylene glycol showed reduction in B.t. Cry protein activity for all counting. Urea as a chaotropic material was used at 9 low concentrations to partially unfold and increase the activity of B.t. Cry protein Cry protein against the 2nd instar larvae of S. littoralis. Almost all concentrations used increased B.t Cry protein activity. Highest increases in mortality rates were recorded: (300%) at (0.24g/1) after 2days post treatment, (188% and 44%) after 4 and 7days respectively, at (0.2g/1). In the case of EDTA, all 6 concentrations used (0.08g/1 to 0.8g/1) caused reductions in B.t. Cry protein activity.5.2. Effect of hyDROPhobic materials on B.t. Cryprotein activity:To increase the hyDROPhobicity of B.t. Cry protein, different hyDROPhobic materials have been used during the study. All the hyDROPhobic materials used increased B. t. Cry protein toxicity.Different hyDROPhobic materials with different hyDROPhobicity (benzene, kerosene, cairosol, soybean oil, olive oil, olean oil, cotton seed oil, and emulsifier) in the presence of amphipathic material, have been added to B.t. Cry protein (Dipel-2X; 2.5 g/1) to investigate possible increase in B.t. Cry protein activity related to possible B.t. Cry protein hyDROPhobicity increase.Best results were induced by addition of olive oil at conc. (10m1/1) to Dipel-2X which gave 600, 460, and 300% increase in mortality rates, than those recorded from using Dipel-2X free of any additive, after 2, 4, and 7 days post treatments, respectively. The sequence of addition in this trial : olive oil was mixed with Dipel-2X powder and then water was added without emulsifier . In another trial olive oil was added to water then the emulsifier and finally Dipel-2X. The increase of B.t. Ciy protein activity was slightly less than that of the former trial. Cotton seed oil came in the second in increasing B.t. Cry protein activity where addition of cotton seed oil at (10m1/1) conc. induced 100%, 86% and 47% increase after 2,4 and 7 days counting. Soybean and olean oils at conc.(8m1/1) gave moderate increase on B.t Cry protein activity. The petroleum hydrocarbons benzene , kerosene and cairosol were used at low concentrations because higher concentrations gave good results but with plant leaves phytotoxicity. Benzene at (8m1/1) conc. gave higherincrease in B.L Cry protein activity than cairosol and kerosene at lower concentrations. Conc. ,2,4,6 and lo m1/1 of emulsifier (esterification of polyethylene glycol) gave reduction in B.t. Cry protein activity. Only (8m1/1) conc. induced increase in B.t. Cry protein activity with 83% , 32% , and 17% for 2 , 4 and 7 days counting.5.3. Effect of different salt species with Hofemeisterbehaviour on B.t. Cry protein activity:Different salt species with Hofemeister behaviour have been used to clarify their effect on B.t. Cry protein activity. Those included potassium citrate, potassium carbonate, potassium sulfate, potassium phosphate, calcium phosphate, calcium carbonate, calcium sulfate,

ammonium sulfate, magnesium sulfate, and barium sulfate .In the case of potassium salts: potassium phosphate and potassium sulfate (at low concentrations) induced best results, followed by potassium citrate. Potassium phosphate at (1.6g/1) gave best increase in Bd. Cry protein activity as the mortality percentages among treated larvae reached 250% and 125% increase than control (treated by Dipel only) after 2 and 7days post treatment, respectively, while (0.6g/1) conc. gave 167% increase after 4days post treatment.. Potassium sulfate at (0.4g/1) conc. gave 200% and 63% increase in Bd. Cry protein activity after 2 and 7 days counting ,while (1.6g/1) conc. gave 175% increase after 4 days counting . Potassium citrate induced 110% and 117% increase in B.t. Cry protein activity at (5g/1) after 4 and 7 days counting while (40g/1) conc.gave 71% increase after 2days counting.161Concerning the calcium salts: calcium carbonate gave better increase in B.t. Cry protein activity than calcium phosphate and calcium sulfate. Conc. (2.5g/1) of calcium carbonate induced 143% ,110% and 100% increase in B.t. Cry protein activity against S. littoralis 2nd instar larvae than control (treated with Dipel alone) after 2,4 and 7 days counting, respectively opposed to 40% , 410/0 and 13% increase respectively after treatment by calcium phosphate at 2.5W1.As for calcium sulfate, it caused the lowest increase in B. t. Cry protein activity when mixed at (10g/1 , 20g/1) conc. (6 —55% increase inmortality rate than control), while remaining conc. (1.25, 2.5, 5 and 40 g/1) induced reductions in B.t. Cry protein activity.Ammonium sulfate which is a good stabilizer for proteins induced great reductions in B.t. Cry protein activity at all higher concentrations, 20, 40, 80, 120 and 160 g/l. While, at low concentrations only one conc. (0.625W1) induces markedly increase (75, 63 and 60%) after 2, 4 and 7 days post treatment respectively.Magnesium sulfate concentrations were 1.25, 2.5, 5, 10, and 20 g/1 for one trial and 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 g/1 for another trial. Only concentrations (1.25g/1 and 0.6g/1) gave significant increase in B.t. Cry protein activity. Conc. (0.6g/1) gave better increase than (1.25g/1).Barium sulfate conc. were 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6, 2.0 and 2.2 g/l. Most of concentrations induced increase in B.t. Cry protein activity, the highest was attributed to (0.8W1) conc.(125% and 88% for 4 and 7 days counting).1625.4. Effect of protein cross-linkers on B.t. Cryprotein activity:Wheat flour as a source of gliadin and glutenin proteins with disulfide bonds was added to B.t. Cry protein (Dipel-2X; 2.5g/1) at concentrations 40, 20, 10, 5, and 2.5 g/1 and bioassayed on the 2nd instal larvae of Slittoralis. Data showed that all wheat flour concentrations used reduced B.t. Cry protein activity. For example (40g/1) conc. induced reduction in B.t. Cry protein activity by 25%, 23, and 0% at 2 days, 4 days and 7 days post treatment, respectively. That same wheat flour conc. was used in a separate trial with activation energy (adding boiled water to the mixture). Interestingly that same wheat flour (40g/1) in the presence of activation energy increased B.t. Cry protein activity by 650% than B.t. Cry protein treatment alone at 2 days counting .In order to investigate the stabilities of introduced protein structures, the mixtures obtained were left under laboratory conditions for 7 days before being bioassayed on S. littoralis 2nd ingiar larvae . Data showed that suspension of B.t. Cry protein alone after 7 days incubation period gave 0% mortality after 2 and 4 days counting but the suspension of B.t. Cry protein combined with wheat flour concentrations (40g/1) in the presence of activation energy after 7 days incubation period induced 47 and 71% mortality after 2 and 4 days post treatment.5.5. The contribution of water in B.t. Cry proteinactivity:In order to study the contribution of water on B.t. Cry protein activity, 0.625g of Dipel-2X was suspended in 250 ml water. During one hr. there was a trial of dipping castor bean leaves in B.t Cry protein suspension every 5 minutes forbioassay on the 2nd instar larvae of g littoralis. Obtained data showed differences in mortality percentages by the same concentration of Dipel-2X, but at different periods of incubating Cry protein in water during one hour. Increasing Cry protein conc. 4 times up to 2.5g/250m1 gave higher mortality rate but differences between 2 and 4 days counting were not too far like that at lower concentration, and still there were fluctuations in Cry protein activity every 5 minutes after addition of water.5.5.1. Effect of mode of application on B.t. Cry proteinactivity :The sequence of addition of Dipel-2X, hyDROPhobic material, emulsifier and water has a great deal of effect on Cry protein activity at the same concentration. For example as, the best result was obtained from the sequence : 1+2+3+4 (i.e., cairosol +Dipel-2X +emulsifier +water) which caused 200% increase of B.t. Cry protein activity, while same items in other sequence of addition : 1+4+2+3 (cairosol + water + Dipel-2X + emulsifier) resulted a decrease (-50%) in B.t. Cry protein activity, 2 days after treatment.

Also the sequence of addition : 2 + 4 +1 +3 (Dipel-2X + water + cairosol+ emulsifier from from left to right) gave best result at 4 days counting (157% increase of B.t. Cry protein activity),while sequence of addition : 1 + 4 + 3 + 2 (cairosol + water + emulsifier + Dipel-2X) reduced B. t. Cry protein activity at 4 days counting, (-43%,) .Data showed that addition of Dipel-2X to ammonium sulfate solution decreased the activity of Dipel-2X (-57 and -54%) after 4 and 7 days counting, respectively. While addition of ammonium sulfate to Dipel-2X suspension at the sameconcentrations increased the activity of Dipel-2X (50% and 43%) after 2 and 4 days counting, respectively.Data showed differences in % mortality obtained by the same concentration of Dipel 2X, but at different periods of incubating Cry protein in water during one hour.Increasing Cry protein conc. 4 times up to2.5g/250m1 gave higher mortality but differences between 2 and 4 days counting were not too far like that at lower concentration and still there fluctuations in Cry protein activity every hr. for airs. bioassay period.The sequence of addition of Dipel 2X, hyDROPhobic material, emulsifier and water has a great deal of effect on Cry protein activity at the same concentration. For example the best result obtained from the sequence : 1+2+3+4 (cairosol +Dipel2X +emulsifier +water from left to right) which gave 200% increase of B.t. Cry protein activity, while same items in other sequence of addition: 1+4+2+3 (cairosol + water + Dipel2X + emulsifier from left to right) gave decrease (-50%) in B.t. Cry protein activity at 2 days counting.5.6. The contribution of activation energy in B. t.Cry protein activity:Boiled water (as a source of activation energy) was added to B.t. Cry protein Cry protein (Dipel-2X;100) alone or combined with additives. The experiments were performed one hour later after suspensions temperature was turned down to room temperature. There was an experiment every one hour up to 6 hrs. for each suspension. The suspensions were left under laboratory conditions for 7 days when other experiments were performed.Data showed that the activity of Dipel-2X (10g/1) against S.littoralis 2nd instar larvae decreased by adding boiled water, i.e. in the presence of activation energy.Combinations of B.t. Cry protein (Dipel-2X) at conc. (10g/1) with additives in the presence of activation energy showed different activities. For each combination, the activity against treated larvae varied according to incubation periods where: ethylene glycol(20m1/1), ethylene glycol (10m1/1) + propylene glycol(10m1/1), glycerol(20m1/1), and olive oil (10m1/1) decreased B.t. Cry protein activity (average mortality rates were : 86.30, 70.06, 85.07 and 63.04% respectively). On the contrary, boric acid (10g/1), yeast(10g/1), and sucrose(20g/1), increased B.t. Cry protein activity (average mortality rates were: 100, 93.04 and 92.22% respectively). While the average mortality rate of B. t. Cry protein alone (in room temperature water) against S.littoralis 2nd instar larvae was 89.68% opposed 59.42% induced by B.t. Cry protein in the presence of activation energy.Data showed that stability of Cry protein structures induced by leaving the mixtures for 7 days incubation period under laboratory conditions, was correspondent to mortality rates by different mixtures where (Dipel-2X; 10g/1) alone induced 10% mortality, (Dipel-2X;10g/1) in the presence of activation energy induced 17%, while the best results were obtained with boric acid(100) (66% mortality), ethylene glycol (10m1/1) + propylene glycol (10m1/1) (64% mortality), followed by olive oil (10m1/1) (54% mortality), glycerol (20m1/1) and sucrose (20g/1) which gave the same activity (31% mortality), ethylene glycol (10m1/1) (22% mortality), while lowest activity was obtained by mixing Dipel with yeast(10g/1) as this mixture caused 7% mortality among treated larvaeTo investigate the effect of activation energy on the activity of Bacillus thuringiensis insecticidal toxins, boiled water was added to B. t. Cry protein (Dipel 2X) powder alone or combined with different additives used during the study. For the B. t. Cry protein (Dipel 2X) powder alone there was increase of activity only when the source of (Dipel 2X) powder was new in closed package. Combinations of B.t. Cry protein (Dipel 2X) at conc. (10g/1) with additives in the presence of activation energy showed different activities. For each combination there were fluctuations in activity according to incubation periods. Ethylene glycol(20m1/1) , ethylene glycol (10m1/1) + propylene glycol(10m1/1), glycerol(20m1/f), and olive oil (10m1/1) decreased B.t. Cry protein activity by different values. On the other hand boric acid(10g/1), yeast(10g/1), and sucrose(20g/1), increased B.t. Cry protein activity by different values.5.7. Effect of protein-protein interaction on B.t.Cry protein activity:Protein concentrations (10g/1), (5g/1), (2.5g/1), (1.25g/1), (0.625g/1) and (0.31g/1) from each of yeast (Table, 7.1) and dried meat (Table 7.2) were mixed with B.t. Cry protein (Dipel-2X) at conc. (2.5g/1) to evaluate possible protein — protein interactions and

its influence on B.t. Cry protein activity. All concentrations used caused increases in B.t. Cry protein activity except only one concentration of yeast at (0.31g/1) which caused decrease in B.t. Cry protein activity (-38%). The best results among other yeast concentrations were obtained with (100) conc. which caused increase in B.t. Cry protein activity (175%, 100%, and 42%) at 2, 4, and 7days post-treatment. The best results among dried meat concentrationswere obtained with (0.31g/1) conc. which caused 46.67% mortality among treated larvae (6000/0 increase in B.t. Cry protein activity than the 6.67/0 resulted by using Dipel alone at 2 days post treatment and (5g/1) conc. which caused 360% increase (79.31% mortality opposed to 17.24% by using Dipel alone at 4 days post-treatment.To test the combined action of kosmotropic material (sucrose) and hyDROPhobic material (olive oil) on protein -protein interactions, a mixture of 2.5g yeast, 2.5g dried meat, 5g sucrose and 2.5 ml olive oil were combined together in 245 ml water, then 5m1 emulsifier was added. Concentrations from this mixture (10, 20, 30, 40, 50 and 60 ml) were added to 0.625g Dipel-2X and water was added until reaching a final volume 250 ml. All concentrations induced variable rates of increase in B.t. Cry protein activity. Highest increase in mortality percentage than control (240%) was obtained after 2 days of feeding S.littoralis 2nd instar larvae on castor bean leaves treated with mixture of 160 ml./1. This mixture caused 56.67% mortality among treated larvae opposed to 16.67%mortality among those treated with Dipel alone. This was followed by 220% increase from the mixture of 120m1./1 at 2 days post-treatment . At 4 days post-treatment, the highest mortality percentage (96.43%) resulted from the mixture of 120m1./1 , followed by 92.86% mortality caused by the concentration 160m1./1. The two mentioned concentrations caused, also, highest mortality percentage (96.43%) at 7days post-treatment, opposed to 50% by dipping the castor bean leaves in the suspension of Dipel-2X alone in water at concentration 2.5g./1.5.8. The introduction of molten globule structurenecessary for pore forming :Wheat flour as a source of gliadin and glutenin proteins with disulfide bonds was added to Bd. Cry protein (Dipel-2X; 2.5g/1) at concentrations 40, 20, 10, 5, and 2.5 g/1 and bioassayed on the 2nd instar larvae of S.littoralis. Data in (Table 4.1.) show that all wheat flour concentrations used reduced B.t Cry protein activity(-25 to -100, -23 to -100 and 0 — 85 % at 2, 4 and 7 days post treatment respectively). Data showed that adding of boiled water to the mixture Dipel-2X; 2.5g11 + wheat flour at 40 & 20 g/l, greatly, increased B.t. Cry protein activity against 2nd instar larvae of S.littoralis (650 & 275, 107 & 79 and 115 & 100% increase in mortality rates compared to mortality percentages recorded by using Dipel alone + boiled water at 2 , 4 and 7 days post-treatment, respectively). On the contrary all the three remaining wheat flour concentrations (10, 5 and 2.5g/1) caused different rates of reduction in B. t. Cry protein activity, (- 50, 0 & 0; - 64 , - 79 & - 50 and — 46, -77 & -46 % after 2 , 4 & ’7 days post-treatment, respectively).To test stability of different B.t. Cry proteinstructures induced in previous experiments all suspensions were left under laboratory conditions for 4days before bioassay on 2nd instar larvae of S.littoralis. The results tabulated in (Table 8.2) show while there was no mortality with the 4 days incubated Bd. Cry protein alone suspension , all remaining suspensions of B.t. Cry protein mixed with wheat flour concentrations in the presence of activation energy and incubated under laboratory conditions gave mortalities ranged from best result (with wheat flour conc.20g/1 which gave 40% and 71% mortalities after 2 and 4 days counting respectively, followed by 40g/1 conc. whichgave 43% and 61% mortalities after 2 and 4 days countingrespectively) to moderate mortality with 10g/1 conc. and low mortality with 5 and 2.5g/1.Data obtained confirm on the effect of adding wheat flour concentrations to Dipel-2X in the presence of activation energy on 2nd instar larvae of S.littoralis. It appears that the wheat flour concentration that should be added to Dipel-2X lie between 40g/1 and 16g/I as those increases in mortality % ranged from 57 to 650% compared to that caused by Dipel-2X alone + activation energy.in an attempt to increase Bd. Cry protein activity by adding [magnesium sulfate (1.25g/1) alone or combined with ethylene glycol (10m1/1) together to Dipel-2X with wheat flour conc. (28, 24, 20, 16 and 12 g/1) in the presence of activation energy . Data show that in case of addition of two additives there was increase in B.t. Cry protein activity for one wheat flour concentration only (28g/1) 73%, 100% and 100% after 2, 4 and 7 days counting post treatments respectively, compared to mortality rates by Dipel-2X + wheat flour (28g/1) + activation energy which were 57%, 89% and 97% after 2, 4 and 7 days counting post treatments respectively.To evaluate effects of kosmotropic,

chaotropic, hyDROPhobic materials and good stabilizer from Hofmeister series on B.t. Cry protein in the presence of wheat flour and activation energy, [(ethylene glycol concentrations: 80, 60, 40, 32, 24, 16, 8 and 4 m1/1), (urea conc. : 40, 20, 10, 5, 2.5, 1.25, 0.625 and 0.31g/1), (benzene conc. : 8, 6, 4, 2 and 1 m1/1) and (ammonium sulfate conc. : 80, 60, 40, 32, 24, 16, 8 and 4 g/1)] were used with Dipel-2X (2.5g/1) + 20g /1 wheat flour + activation energyFor ethylene glycol, there was a slight increase in B.t. Cry protein activity at conc.32m1/1 which gave 60% and 96% mortality compared with 50% and 86% mortality obtained with Dipel-2X (2.5g/1) + 20g /1 wheat flour + activation energy after 2 and 4 days counting post treatments respectively.For urea, there were two concentrations (20 and 0.3 lgfl) that gave highest mortality (100% mortality) after 4 days post treatments.For benzene, highest mortality (100% mortality) occurred after 4 days counting obtained by (2 ml/1). Other benzene conc. gave same mortality rates of Dipel-2X (2.5g/1) + 20g /1 wheat flour + activation energy after 4 days counting, but decreased at 2 days counting. On the other hands all ammonium sulfate conc. used caused reductions in B.t. Cry protein activity.At the end of experiment program there was a last experiment to evaluate at one time effects of all additives (at conc. gave best result for each) on the activity of B.t. Cry protein activity(Dipe1-2X. 2.5g/1) on 2nd instar larvae of S.littoralis. Interestingly. ,there was no activity of Dipel-2X alone or combined with all additives except for boric acid (10g/1) which gave 40%, 50% and 50% mortality after 2, 4 and 7 days counting post treatments. After using activation energy + 20W1 wheat flour, mortality rates of mixture with boric acid increased up to 93%, 97% and 100% after 2, 4 and 7 days counting post treatments, respectively. For other additives there were increase of B.t. Cry protein activity ranged between 40% and 50% mortality for calcium sulfate, glycerol and ethylene glycol after 4 days counting. All remaining additives gave less increase.The same experiment has been performed using another source of B.t. Cry protein (Dipel-2X powder) in newclosed package. Interestingly, there was no activity of Dipel-2X alone or combined with all additives except for olive oil (8 g/l) which gave 50 and 67 % mortality after 2 and 4 days counting post treatments. All other additives gave 0 — 10% mortality for 2days counting. It should be mentioned that boric acid used in this experiment was at conc. (5g/1) instead of (10g/1) used in previous experiment. Also olive oil was not used in previous experiment. After using activation energy + 20g/1 wheat flour with B.t. Cry protein (Dipel-2X powder) and all the assayed additives, there were much increase in mortality rates in all treatments. Just the presence of activation energy with Dipel-2X powder increased mortality from zero up to 93, 93 and 93% after 2, 4 and 7days counting. Adding wheat flour (20g/1) increased mortality up to 97% and 97% after 4 and 7 days counting. For remaining additives, potassium phosphate (1.6g/1) gave 100% mortality for the mixture after 2 days counting. Additives gave 100% mortality after 4 days counting were: sucrose (20g/1), ethylene glycol + probylene glycol (20g/1), cairosol (2m1/1), olive oil (8 m1/1), PEG (20g/1), benzene (2m1/1) and kerosene (1m1/1) (Table, 8.13).5.9. Effect of boric acid concentrations on B.t. Cryprotein activity:Boric acid concentrations: 40, 20, 10. 5, 2.5 and 1.25 g/1 were mixed with (Dipel-2X; 2.5g/1). Data obtained showed that boric acid at (20g/1) increased mortality of mixture up to 100% at 2 days counting compared with 38% mortality induced by Dipel-2X alone . Also (10g/1) conc. of boric acid increased mortality of mixture up to 100% at 7 days counting compared with 63% mortality induced by Dipel-2X alone. Lowconcentration of boric acid (1.25g/1) reduced B.t. Cry protein activity (- 80%, - 41% and -30%) at 2, 4 and 7 days post treatments, respectively.