

ANEXO I:

Biological tests

The applicability of compounds of formula (I) alone or in combination with other active ingredients in application methods according to the present invention may be evaluated in test examples as provided herein below or in similar assays. These test examples are not to be construed in any way as limiting.

As mentioned above, mixtures of compound of formula (I) with other agriculturally active ingredients may show surprisingly synergistic effects, which can also be demonstrated in the biological test systems described below.

Synergism can be described as an interaction where the combined effect of two or more compounds is greater than the sum of the individual effects of each of the compounds. The presence of a synergistic effect in terms of percent control, between two mixing partners (X and Y) can be calculated using the Colby equation (Colby, S. R., 1967, Calculating Synergistic and Antagonistic Responses in Herbicide Combinations, Weeds, 15, 20-22):

$$E = X + Y - \frac{XY}{100}$$

When the observed combined control effect is greater than the expected combined control effect (E), then the combined effect is synergistic.

The analysis of synergism or antagonism between the mixtures or compositions was determined using Colby's equation.

B.1 Control of animal pests

With regard to the control of animal pests affecting plant propagation material, especially seeds, the following test systems and results demonstrate the pesticidal activity of the carboxamide compound of formula I alone and its synergistic pesticidal activity in combination with other insecticidal active ingredients.

Test B.1.1 Control of Wireworm (*Melanotus communis*)

For evaluating control of wireworm (*Melanotus communis*) through direct contact method, the individual insects were dipped directly into compound solution.

The compounds or mixtures were dissolved in acetone at different concentrations. The compounds or mixtures were formulated using a solution containing 50% (v/v) acetone in water with 0.02 wt% Kinetic®.

For the experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations. Wireworms were dipped directly into solution for three seconds and then allowed to air dry on filter paper.

About 11 cm³ of water-moistened loamy sand was dispensed into each 16-cm² cell of a 32-cell rearing tray. One treated wireworm larva was infested into each cell along with two germinating wheat seeds. Cells were then covered with adhesive bio-assay tray lids. Each cell was a replicate and replication was 10x. After infestation, the test was maintained in an incubator at 26 ± 1 °C in the dark. Mortality (dead + moribund insects) was evaluated 3 days after treatment (DAT) and mean percent mortality was calculated relative to the untreated control. The results are listed in table B.1.1.

Table B.1.1:

<i>Melanotus communis</i>	concentration [ppm]	Average Control %
Test B.1.1 a):		
Carboxamide compound of formula 1	3	33
Cyantraniliprole	225	22
Cyantraniliprole + Carboxamide compound of formula 1	225+3	78*
Test B.1 .1 .b)		
Carboxamide compound of formula I	3	33
Emamectin	800	11
Emamectin + Carboxamide compound of formula I	800+3	44*
Test B.1.1.c)		
Carboxamide compound of formula I	3	33
Fipronil	30	22
Fipronil + Carboxamide compound of formula I	30+3	89*
Test B.1.1.d)		
Carboxamide compound of formula I	3	33
Thiamethoxam	25	11
Thiamethoxam + Carboxamide compound of formula I	25+3	44*

*synergistic control effect according to Colby's equation

Test B.1.2 Control of Western Corn Rootworm (*Diabrotica virgifera virgifera*)

For evaluating control of western corn rootworm (*Diabrotica virgifera virgifera*) through maximum exposure method, the insects were exposed to treated soil.

The compounds or mixtures were first dissolved in acetone, and then mixed with soil to obtain the desired different ppm concentrations (w/w) of compound/soil or mixture/soil. For experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations. Treatments were applied in solution to sifted (#10 sieve) loamy sand in a plastic bag. Treatments were thoroughly incorporated by sealing and shaking each bag by hand and allowing the solution to soak through the soil mass for at least 10 minutes. Bags were then unsealed and kept open in a fume hood overnight to evaporate the solvent from the soil. One day after treatment (DAT) water for moisture and water-soaked millet seed as a food source were added to each bag and incorporated thoroughly. About 11 cm³ of the millet and soil mixture was dispensed into a 1-oz. plastic cup. Each cup was infested with 10 western corn rootworm second-instar larvae and covered. Each cup was a replicate and replication was 3x. The test was maintained in an incubator at 26 ± 1 °C in the dark. Mortality (dead + moribund insects) was evaluated 3 days after infestation (DAI) and mean percent mortality was calculated relative to the untreated control. The results are listed in table B.1.2.

Table B.1,2:

<i>Diabrotica virgifera</i>	concentration compound mixture/soil]	[ppm or	Average Control %
Test B.1.2.a)			
Carboxamide compound of formula I	0.01		30
Clothianidin	0.1		33
Clothianidin +	0.1 +0.01		70*

Carboxamide compound of formula I		
Test B.1.2.b)		
Carboxamide compound of formula I	0.01	30
Cyantraniliprole	3.1	23
Cyantraniliprole + Carboxamide compound of formula I	3.1+0.01	50*
Test B.1.2.c)		
Carboxamide compound of formula I	0.01	30
Fipronil	0.03	27
Fipronil + Carboxamide compound of formula I	0.03+0.01	63*
Test B.1.2.d)		
Carboxamide compound of formula I	0.01	30
Imidacloprid	0.13	17
Imidacloprid + Carboxamide compound of formula I	0.13+0.01	63*
<i>Diabrotica virgifera virgifera</i>	concentration compound mixture/soil] [ppm or	Average Control %
Test B.1.2.e)		
Carboxamide compound of formula I	0.01	30
Thiacloprid	0.2	37
Thiacloprid + Carboxamide compound of formula I	0.2+0.01	70*

*synergistic control effect according to Colby's equation

Test B.1.3 Control of Black Cutworm (*Agrotis ipsilon*)

For evaluating control of black cutworm (*Agrotis ipsilon*) through direct contact method, the individual insects were dipped directly into compound solution.

The compounds or mixtures were dissolved in acetone at different concentrations. The compounds or mixtures were formulated using a solution containing 50% (v/v) acetone in water with 0.02 wt% Kinetic®. For experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations. Second-instar black cutworms were dipped directly into solution for three seconds and then allowed to air dry on filter paper. One treated black cutworm larva was infested into each 16-cm² cell of a 32-cell rearing tray along with the excised shoot of a corn plant and a moistened cotton wick. Cells were then covered with adhesive bio-assay tray lids. Each cell was a replicate and replication was 16x. After infestation, the test was maintained in an incubator at 25.5 ± 1 °C and a 14L:10D light cycle. Mortality (dead + moribund insects) was evaluated 1 and 5 days after treatment (DAT) and mean percent mortality was calculated relative to the untreated control. The results are listed in table B.1.3.

Table B.1.3:

<i>Agrotis ipsilon</i>	concentration [ppm]	Average Control % 1 DAT	Average Control % 5 DAT
Test B.1.3.a)			
Carboxamide compound of formula I	3	38	44
Fipronil	300	13	31
Fipronil + v	300+3	81*	81*

*synergistic control effect according to Colby's equation

Test B.1.4 Control of seedcorn maggot (*Delia platura*)

For evaluating control of seedcorn maggot (*Delia platura*) through maximum exposure, the insects were exposed to treated soil.

The compounds or mixtures were first dissolved in acetone, and then mixed with soil to obtain the desired different ppm concentrations (w/w) of compound/soil or mixture/soil. For experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations. Treatments were applied in solution to dry, sifted (#10 sieve) play sand in a glass jar. Treatments were thoroughly incorporated by capping and shaking each jar by hand and allowing the solution to soak through the sand mass for at least 10 minutes. The jars were then uncapped and the sand was kept in a fume hood for 4 hours to evaporate the solvent. After the sand was dry, 10 cm³ of the treated sand was dispensed into a 1-oz. plastic cup with bone meal in the bottom as a food source. Each cup was moistened with 4 ml water, infested with five seedcorn maggot second-instar larvae, and covered. Each cup was a replicate and replication was 5x. The test was maintained in an incubator at 22 ± 1 °C in the dark. Mortality (dead + moribund insects) was evaluated 2 days after treatment (DAT) and mean percent mortality was calculated relative to the untreated control. The results are listed in table B.1 .4.

Table B.1.4

<i>Delia platura</i>	Concentration compound mixture/soil]	[ppm or	Average Control %
Carboxamide compound of formula I	0.06		21
Cyantraniliprole + Carboxamide compound	10+0.06		96*

of formula I		
Test B.1.4.b)		
V	0.06	21
Imidacloprid	3	18
Imidacloprid + Carboxamide compound of formula I	3+0.06	52*

*synergistic control effect according to Colby's equation

Test B.1.5 Control of root-knot nematodes (*Meloidogyne spp.*)

For evaluating control of root-knot nematodes (*Meloidogyne spp.*) through exposure via drench application, the nematodes were introduced to treated cucumber plants. The compounds or mixtures were dissolved in acetone at different concentrations. The compounds or mixtures were formulated using a solution containing 50% (v/v) acetone in water with 0.02 wt% Kinetic®. For experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations.

Germinated cucumber seeds were planted in 1-oz black plastic cups (one seed per cup) with dry, sifted (#10 sieve) play sand. Treatments were applied as a drench in 7 ml solution to each cup which were held in a fume hood for 1 hour to allow acetone to evaporate. The cups were moved to an environmental chamber (25 ± 2 °C, 24L) and watered daily for the duration of the test. Each cup was a replicate and replication was 5x. At the cucumber's cotyledon stage, each cup was infested with 500 root-knot nematode juveniles (J2s) in 1 ml distilled water.

Four weeks after infestation, cucumber roots were washed off and galls and egg masses were counted. Control was calculated as the reduction in galls or egg masses relative to

the untreated control. Mean percent control was calculated for each treatment. The results are listed in table B.1 .5.

Table B.1.5:

<i>Delia platura</i>	concentration [mg compound or mixture/plant]	Average Gall Control %	Average Egg Mass Control %
Test B.1.5.a)			
Carboxamide of compound formula I	0.3	35	-21
Abamectin	0.017	40	39
Test B.1.5.b)			
Carboxamide of compound formula I	0.3	35	-21
Abamectin	0.008	24	17
Abamectin + Carboxamide of compound formula I	0.008+0.3	100*	99*

*synergistic control effect according to Colby's equation

Further examples of assays with which the control of soil infesting pests may be evaluated described in the following.

BP.1.1 Assays for spider mites (*Tetranychus*)

Test BP.1.2 Soil drench assay in Lima bean

Test solution comprising a compound of the present invention is prepared at desired concentration using water and an organic solvent. Potted lima bean plants are treated

with test solution by means of soil drenching. The test compounds are applied as a soil drench in 2 ml of solution at the rate of 4 mg active ingredient/plant (2mg/ml). Technical material is dissolved in acetone, and distilled water was added to achieve a final concentration of 5% acetone. Four days after treatment, a mixed population of two spotted spider mites (*Tetranychus urticae*) is released onto the leaves. After infestation, plants are kept on a light cart in the laboratory and top watered daily.

Five days after the release of spider mites, the acaricidal efficacy is measured by means of the rating of the damage caused by spider mites or the spider mite mortality: the number of TSSM are counted on plants, percent damage as lesions is visually assessed and means are calculated for each treatment. Mean percent population reduction relative to the solvent blank control is calculated. Mean percent reduction in damage relative to the solvent blank is calculated as $100 - (\text{Mean\% lesions in treatment} / \text{Mean \% lesions in Solvent blank}) * 100$.

Test BP.1.3 Seed treatment assay in Cotton

Test solution comprising a compound of the present invention is prepared at desired concentration using water and an organic solvent. Cotton seeds are coated with such prepared test solution at the rate 0.5 mg active ingredient /seed and sown to the pots. After plant emergence, a mixed population of two spotted spider mites is released onto the leaves.

Four days after the release of spider mites, the acaricidal efficacy is measured by means of the rating of the damage caused by spider mites or the spider mite mortality: percent damage as lesions was visually assessed and means are calculated for each treatment.

Mean percent reduction in damage relative to the solvent blank is calculated as $100 - (\text{Mean\% lesions in treatment} / \text{Mean \% lesions in Solvent blank}) * 100$.

Test BP.1.4 Seed treatment assay in Cucumber

Test solution comprising a compound of the present invention is prepared at desired concentration using water and acetone as organic solvent. Cucumber seeds are coated with such prepared test solution applied at the rate of 0.5 mg active ingredient /seed and sown to the pots. After plant emergence (eleven days after treatment & planting), a mixed population of two spot- ted spider mites is released onto the leaves.

Four days after the release of spider mites, the acaricidal efficacy is measured by means of the rating of the damage caused by spider mites.

Percent damage as lesions is visually assessed, and means are calculated for each treatment. Mean percent damage reduction relative to the solvent blank is calculated as $100 - (\text{mean\% lesions in treatment} / \text{mean \% lesions in Solvent blank}) * 100$.

BP.1.5 Assays for nematode (*Meloidogyne*)

Test B.2.1 Tomato soil drench against root-knot nematode juveniles

Tomatoes are grown in potting soil until the approximate first true leaf stage (about 2 weeks after planting). Tomatoes are transplanted into play sand. Seven days after transplant, technical material of test compound is dissolved in acetone, and then water is added to achieve a final concentration of 50% acetone. 1 ml. of solution is pipetted onto the tomato root zone. One day after treatment (DAT), each pot is infested with about 500

root-knot nematode (*Meloidogyne spp.*) juveniles in 1 ml distilled water. Immediately after infestation, plants are placed in the greenhouse. Plants are top watered and fertilized daily. At 14 DAT, the tomato roots are rinsed off, and the number of galls is counted. Replication is 5-times.

B.2 Control of phytopathological fungi

With regard to the control of phytopathological fungi affecting plant propagation material, especially seeds, the following test systems and results demonstrate the fungicidal activity of the carboxamide compound of formula I alone, and its synergistic fungicidal activity in combination with other fungicidal active ingredients.

Microtests for the evaluation of fungicidal activity

The active compounds were formulated separately as a stock solution having a concentration of 10,000 ppm in dimethyl sulfoxide.

B.2.1. Activity against rice blast *Pyricularia oryzae*

The stock solutions were mixed according to the indicated ratio, pipetted onto a micro titer plate (MTP) and diluted with water to the stated concentrations. A spore suspension of *Pyricularia oryzae* in an aqueous biomalt or yeast-bactopeptone-glycerine solution was then added. The plates were placed in a water vapor-saturated chamber at a temperature of 18°C. Using an absorption photometer, the MTPs were measured at 405 nm 7 days after the inoculation. The results are given in table B.2.1 hereinbelow.

Table B.2.1.: *Pyricularia oryzae*

Active compound / active compounds in mixture	Concentration (ppm)	Mixture (ratio)	Observed efficacy	Calculated efficacy according to Colby (%)	Synergism (%)
carboxamide compound of formula I	16 4 1 0.063	- - - -	4 15 12 2		
Epoxiconazol	0,25 0.063	- -	28 1		
carboxamide compound of formula I Epoxiconazol	16 0.25	64:1	100	31	69
carboxamide compound of formula I Epoxiconazol	4 0.063	63:1	100	15	85
carboxamide compound of formula I Epoxiconazol	4 0.25	16:1	100	39	61
carboxamide compound of formula I Epoxiconazol	1 0.063	16:1	100	13	87

B.2.2. Activity against early blight caused by *Alternaria solani*

The stock solutions were mixed according to the indicated ratio, pipetted onto a micro titer plate (MTP) and diluted with water to the stated concentrations. A spore suspension of *Alternaria solani* in an aqueous biomalt or yeast-bactopeptone-glycerine solution was then added. The plates were placed in a water vapor-saturated chamber at a temperature

of 18°C. Using an absorption photometer, the MTPs were measured at 405 nm 7 days after the inoculation. The results are given in table B.2.2 hereinbelow.

Table B.2.2.: *Alternaria solani*

Active compound / active compounds in mixture	Concentration (ppm)	Mixture (ratio)	Observed efficacy	Calculated efficacy according to Colby (%)	Synergism (%)
carboxamide compound of formula I	63	-	0		
	16	-	0		
	4	-	0		
	1	-	0		
Pyraclostrobin	0.063	-	12		
	0,016	-	0		
Triticonazol	1	-	23		
	0,25	-	0		
carboxamide compound of formula I		254:1	36	12	24
Pyraclostrobin	16				
	0.063				
carboxamide compound of formula I		1000:1	40	12	28
	63				
Pyraclostrobin	0.063				
carboxamide compound of formula I		4000:1	24	0	24
	63				
Pyraclostrobin	0.016				
carboxamide compound of formula I		63:1	43	23	20
Triticonazol	63				
	1				
carboxamide compound of formula I		64:1	32	0	32
	16				
Triticonazol	0.25				
carboxamide compound of		16:1	20	0	20

formula I	4				
Triticonazol	0.25				
carboxamide compound of formula I	4	4:1	52	23	29
Triticonazol	1				
carboxamide compound of formula I	1	4:1	34	0	34
Triticonazol	0.25				

B.2.3. Activity against *Microdochium nivale*

The stock solutions were mixed according to the indicated ratio, pipetted onto a micro titer plate (MTP) and diluted with water to the stated concentrations. A spore suspension of *Microdochium nivale* in an aqueous biomalt or yeast-bactopeptone-glycerine solution was then added. The plates were placed in a water vapor-saturated chamber at a temperature of 18°C. Using an absorption photometer, the MTPs were measured at 405 nm 7 days after the inoculation. The results are given in table B.2.3 hereinbelow.

Table B.2.3.: *Microdochium nivale*

Active compound / active compounds in mixture	Concentration (ppm)	Mixture (ratio)	Observed efficacy	Calculated efficacy according to Colby (%)	Synergism (%)
carboxamide compound of formula I	63	-	4		
	16	-	15		
	4	-	13		
Pyraclostrobin	0.016	-	46		
Fluxapyroxad	0.063	-	0		
	0.004	-	0		
Epoxiconazol	0.016	-	29		
	0.004	-	8		
carboxamide					

compound of formula I Pyraclostrobin	16 0.016	1000:1	74	54	20
carboxamide compound of formula I Pyraclostrobin	63 0.016	4000:1	93	48	45
carboxamide compound of formula I Fluxapyroxad	63 0.063	1000:1	35	4	31
carboxamide compound of formula I Fluxapyroxad	63 0.004	16000:1	25	4	21
carboxamide compound of formula I	4	250:1	63	38	25
Epoxiconazol	0.016				
carboxamide compound of formula I Epoxiconazol	63 0.016	4000:1	99	31	68
carboxamide compound of formula I	16	4000:1	99	21	78
Epoxiconazol	0.004				

B.2.4. Activity against *Rhizoctonia solani*

The stock solutions were mixed according to the indicated ratio, pipetted onto a micro titer plate (MTP) and diluted with water to the stated concentrations. A spore suspension of *Rhizoctonia solani* in an aqueous biomalt or yeast-bactopeptone-glycerine solution was then added. The plates were placed in a water vapor-saturated chamber at a temperature of 18°C. Using an absorption photometer, the MTPs were measured at 405 nm 7 days after the inoculation. The results are given in table B.2.4 hereinbelow.

Table B.2.4.: *Rhizoctonia solani*

Active compound / active compounds in mixture	Concentration (ppm)	Mixture (ratio)	Observed efficacy	Calculated efficacy according to Colby (%)	Synergism (%)
carboxamide compound of formula I	16	-	0		
	4	-	0		
	1	-	0		
Epoxiconazol	0.016	-	24		
	0.004	-	0		
	0.001	-	0		
Triticonazol	0.25	-	41		
carboxamide compound of formula I		250:1	55	24	31
Epoxiconazol	4				
	0.016				
carboxamide compound of formula I		4000:1	96	31	65
Epoxiconazol	63				
	0.016				
carboxamide compound of formula I	16				
Epoxiconazol	0.004				
carboxamide compound of formula I		4000:1	20	0	20
Epoxiconazol	4				
	0.001				
carboxamide compound of formula I		63:1	44	24	20
Epoxiconazol	1				
	0.016				
carboxamide compound of formula I		64:1	67	41	26
Triticonazol	16				
	0.25				
carboxamide compound of formula I		16:1	67	41	26
	4				

Triticonazol	0.25				
carboxamide compound of formula I	1	4:1	69	41	28
Triticonazol	0.25				

The measured parameters were compared to the growth of the active compound-free control variant (100%) and the fungus-free and active compound-free blank value to determine the relative growth in % of the pathogens in the respective active compounds.

These percentages were converted into efficacies.

As mentioned above, the expected efficacies of active compound mixtures were determined using Colby's formula [R.S. Colby, "Calculating synergistic and antagonistic responses of herbicide combinations", Weeds 15, 20-22 (1967)] and compared with the observed efficacies.

ANEXO II

Biological tests

Synergism can be described as an interaction where the combined effect of two or more compounds is greater than the sum of the individual effects of each of the compounds. The presence of a synergistic effect in terms of percent control, between two mixing partners (X and Y) can be calculated using the Colby equation (Colby, S. R., 1967, Calculating Synergistic and Antagonistic Responses in Herbicide Combinations, *Weeds*, 15, 20-22):

$$E = X + Y - \frac{XY}{100}$$

When the observed combined control effect is greater than the expected combined control effect (E), then the combined effect is synergistic.

The following tests demonstrate the control efficacy of compounds, mixtures or compositions of this invention on specific pests. However, the pest control protection afforded by the compounds, mixtures or compositions is not limited to these species. In certain instances, combinations of a compound of this invention with other invertebrate pest control compounds or agents are found to exhibit synergistic effects against certain important invertebrate pests.

The analysis of synergism or antagonism between the mixtures or compositions was determined using Colby's equation.

Biological Examples of the Invention

Test 1

For evaluating control of vetch aphid (*Megoura viciae*) through contact or systemic means the test unit consisted of 24-well-microtiter plates containing broad bean leaf disks.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were sprayed onto the leaf disks at 2.5µl, using a custom built micro atomizer, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, the leaf disks were air-dried and 5 – 8 adult aphids placed on the leaf disks inside the microtiter plate wells. The aphids were then allowed to suck on the treated leaf disks and incubated at $23 \pm 1^{\circ}\text{C}$, $50 \pm 5\%$ RH for 5 days. Aphid mortality and fecundity was then visually assessed. For the mixture tested the results are listed in table 1.

Table 1

Vetch Aphid	ppm	Average Control %
Flupyradifurone + Compound of formula I	0.4 + 0	0
	0 + 0.4	0
	0.4 + 0.4	100*

*synergistic control effect according to Colby's equation

Test 2

For evaluating control of green peach aphid (*Myzus persicae*) through systemic means the test unit consisted of 96-well-microtiter plates containing liquid artificial diet under an artificial membrane.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were pipetted into the aphid diet, using a custom built pipetter, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, 5 – 8 adult aphids were placed on the artificial membrane inside the microtiter plate wells. The aphids were then allowed to suck on the treated aphid diet and incubated at $23 \pm 1^{\circ}\text{C}$, $50 \pm 5\%$ RH for 3 days. Aphid mortality and fecundity was then visually assessed. For the mixture tested the results are listed in table 2.

Table 2

Green Peach Aphid	ppm	Average Control %
Emamectin + Compound of formula I	4 + 0	50
	0 + 0.08	0
	4 + 0.08	100*

*synergistic control effect according to Colby's equation

Test 3

For evaluating control of boll weevil (*Anthonomus grandis*) the test unit consisted of 24-well-microtiter plates containing an insect diet and 20-30 *A. grandis* eggs.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were sprayed onto the insect diet at 20µl, using a custom built micro atomizer, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, microtiter plates were incubated at $23 \pm 1^{\circ}\text{C}$, $50 \pm 5\%$ RH for 5 days. Egg and larval mortality was then visually assessed. For the mixture tested the results are listed in table 3.

Table 3

Boll Weevil	ppm	Average (Control %)
Acetamiprid + Compound of formula I	0.8 + 0	0
	0 + 0.08	0
	0.8 + 0.08	50*

*synergistic control effect according to Colby's equation

Test 4

For evaluating control of tobacco budworm (*Heliothis virescens*) the test unit consisted of 96-well-microtiter plates containing an insect diet and 15-25 *H. virescens* eggs.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were sprayed onto the insect diet at 10µl, using a custom built micro atomizer, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, microtiter plates were incubated at $28 \pm 1^{\circ}\text{C}$, $80 \pm 5\%$ RH for 5 days. Egg and larval mortality was then visually assessed. For the mixture tested the results are listed in table 4.

Table 4

Tobacco budworm	ppm	Average (Control %)
Thiacloprid + Compound of formula I	2 + 0	0
	0 + 0.4	50
	2 + 0.4	100*

*synergistic control effect according to Colby's equation

Test 5

For evaluating control of yellow fever mosquito (*Aedes aegypti*) the test unit consisted of 96-well-microtiter plates containing 200µl of tap water per well and 5-15 freshly hatched *A. aegypti* larvae.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were sprayed onto the insect diet at 2.5µl, using a custom built micro atomizer, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, microtiter plates were incubated at $28 \pm 1^{\circ}\text{C}$, $80 \pm 5\%$ RH for 2 days. Larval mortality was then visually assessed. For the mixture tested the results are listed in table 5.

Table 5

Yellow Fever Mosquito	ppm	Average (Control %)
Thiacloprid + Compound of formula I	2 + 0	0
	0 + 0.4	50
	2 + 0.4	75*

*synergistic control effect according to Colby's equation

Test 6

For evaluating control of tobacco budworm (*Heliothis virescens*) the test unit consisted of 96-well-microtiter plates containing an insect diet and 15-25 *H. virescens* eggs.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were sprayed onto the insect diet at 10µl, using a custom built micro atomizer, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, microtiter plates were incubated at $28 \pm 1^{\circ}\text{C}$, $80 \pm 5\%$ RH for 5 days. Egg and larval mortality was then visually assessed. For the mixture tested the results are listed in table 6.

Table 6

Tobacco budworm	ppm	Average (Control %)
Tioxazafen + Compound of formula I	80 + 0	0
	0 + 0.16	0
	80 + 0.16	50*

*synergistic control effect according to Colby's equation

Test 7

For evaluating control of yellow fever mosquito (*Aedes aegypti*) the test unit consisted of 96-well-microtiter plates containing 200µl of tap water per well and 5-15 freshly hatched *A. aegypti* larvae.

The compounds or mixtures were formulated using a solution containing 75% water and 25% DMSO. Different concentrations of formulated compounds or mixtures were sprayed onto the insect diet at 2.5µl, using a custom built micro atomizer, at two replications.

For experimental mixtures in these tests identical volumes of both mixing partners at the desired concentrations respectively, were mixed together.

After application, microtiter plates were incubated at $28 \pm 1^{\circ}\text{C}$, $80 \pm 5\%$ RH for 2 days. Larval mortality was then visually assessed. For the mixture tested the results are listed in table 7.

Table 7

Yellow Fever Mosquito	ppm	Average (Control %)
Tioxazafen + Compound of formula I	16 + 0	0
	0 + 0.16	0
	16 + 0.16	50*

*synergistic control effect according to Colby's equation

Test 8

For evaluating control of seedcorn maggot (*Delia platura*) through maximum exposure, the insects were exposed to treated soil.

The compounds or mixtures were first dissolved in acetone, and then mixed with soil to obtain the desired different ppm concentrations (w/w) of compound/soil or mixture/soil. For experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations. Treatments were applied in solution to dry, sifted (#10 sieve) play sand in a glass jar. Treatments were thoroughly incorporated by capping and shaking each jar by hand and allowing the solution to soak through the sand mass for at least 10 minutes. The jars were then uncapped and the sand was kept in a fume hood for 4 hours to evaporate the solvent.

After the sand was dry, 10 cm³ of the treated sand was dispensed into a 1-oz. plastic cup with bone meal in the bottom as a food source. Each cup was moistened with 4 ml water, infested with five seedcorn maggot second-instar larvae, and covered. Each cup was a replicate and replication was 5x. The test was maintained in an incubator at 22 ± 1 °C in the dark. Mortality (dead + moribund insects) was evaluated 2 days after treatment (DAT) and mean percent mortality was calculated relative to the untreated control. The results are listed in table B.1.4.

Table 8

<i>Delia platura</i>	ppm	Average Control %
Acetamiprid + Compound of formula I	0.4 + 0	12
	0 + 0.3	8
	0.4 + 0.3	33* (expected: 33)

*synergistic control effect according to Colby's equation

Test 9Control of Black Cutworm (*Agrotis ipsilon*)

For evaluating control of black cutworm (*Agrotis ipsilon*) through direct contact method, the individual insects were dipped directly into compound solution.

The compounds or mixtures were dissolved in acetone at different concentrations. The compounds or mixtures were formulated using a solution containing 50% (v/v) acetone in water with 0.02 wt% Kinetic®. For experimental mixtures, identical volumes of both mixing partners were mixed together to achieve the desired respective concentrations. Second-instar black cutworms were dipped directly into solution for three seconds and then allowed to air dry on filter paper.

One treated black cutworm larva was infested into each 16-cm² cell of a 32-cell rearing tray along with the excised shoot of a corn plant and a moistened cotton wick. Cells were then covered with adhesive bio-assay tray lids. Each cell was a replicate and replication was 16x. After infestation, the test was maintained in an incubator at 25.5 ± 1 °C and a 14L:10D light cycle. Mortality (dead + moribund insects) was evaluated 1 and 5 days after treatment (DAT) and mean percent mortality was calculated relative to the untreated control. The results are listed in table B.1.3.

Table 9:

<i>Agrotis ipsilon</i>	concentration [ppm]	Average Control % 1 DAT
Carboxamide compound of formula I	2	0
Emamectin	15	6
Emamectin +Compound of formula I	15+2	13* (expected: 6)

*synergistic control effect according to Colby's equation

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