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EFFECT OF GAMMA RADIATION ON THE TOXICITY OF MILBEMECTIN AND CHLORFENAPYR IN ACARICIDE RESISTANT AND SUSCEPTIBLE STRAINS OF *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE)

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ABSTRACT

The spider mite *Tetranychus urticae* Koch is considered one of the most important phytophagous mites, causing considerable damage in several agricultural crops. The aim of this study was to evaluate the effect of gamma radiation on the toxicity of the acaricides milbemectin and chlorfenapyr in resistant and susceptible strains of *T. urticae*. The R and S strains for milbemectin and chlorfenapyr were irradiated with gamma radiation at Gammacell-220 source at doses of 5, 10, 20, 40 e 80 Gy. Five concentrations of milbemectin and chlorfenapyr were evaluated, making applications 24 hours after irradiation. Mites of the controls were sprayed with the same acaricide concentrations used for the R and S strains but they were not exposed to gamma radiation. Experiments on the effects of gamma radiation on the growth rates of mites for acaricide resistant and susceptible strains of *T. urticae* were also carried out. Tests with the Milbemectin S strain showed an increased susceptibility to the acaricide milbemectin, when the mites were irradiated (20 Gy), in comparison with the control (non irradiated mites). For the Milbemectin R strain, there was no significant influence of gamma irradiation on the toxicity of milbemectin to the mites of this strain. For the Chlorfenapyr S strain, the effect of gamma radiation was similar to that observed for Milbemectin S strain, with increased toxicity of chlorfenapyr to the mites of this susceptible strain. In the case of the Chlorfenapyr R strain, the mites exposed to gamma radiation showed to be more tolerant to chlorfenapyr, considering the LC_{10} values. The same trend was observed for the LC_{50} values, however, there was no significant difference with the control. The experiments showed that doses of 200 and 300 Gy eliminated the mite populations of acaricide resistant and susceptible strains of *T. urticae*, in a period of ten days. The dose of 100 Gy did not lead to total elimination of the mite populations, but reduced significantly the egg viability of *T. urticae*. The highest effect was observed for the Chlorfenapyr S strain, for which the instantaneous growth rate (r_i)of irradiated mites was 3.8 times lower than that of the control. The effect of gamma radiation was lower for the Chlorfenapyr R strain, with the r_i value 40% higher than that of the Chlorfenapyr S strain.

1. INTRODUCTION

Brazil is considered one of the largest producers of ornamental plants in the world [15,1]. It is estimated that this sector accounts for the generation of 3.7 direct jobs per hectare [12]. Netherlands and the USA remain the most important trade partners of Brazilian floriculture among 38 destination countries in 2007, accounting for 78.4% of Brazilian exports in the sector [14]. However, this large trade in plants may pose risks to importing countries, due to the introduction of non-indigenous species, resulting in potential economic losses. According to [5], eleven mite families were identified in 12 plant shipments arriving via air cargo from Guatemala, Honduras, and Costa Rica to Miami International airport in Florida in 2003. The intensive use of pesticides for the control of pest mites such as the two-spotted spier mite, *Tetranychus urticae* Koch, has caused the development of acaricide resistance in populations of these mites [19]. Methods of eliminating pests in post-harvest flowers were discussed by several authors [10,20,11]. The use of gamma radiation in the treatment of ornamental plants can be a useful tool for the control of *T. urticae*. However many species of plants are susceptible to radiation. The combined use of agrochemicals and gamma radiation can be an alternative to reducing the dose of radiation and for the preservation of the irradiated material. Studies on the radiosensitivity of acaricide resistant and susceptible mites are still very rare, and this is another aspect that needs to be solved. The objective of this study was to evaluate the effect of gamma radiation on the toxicity of the acaricides milbemectin and chlorfenapir in susceptible and resistant strains of *T. urticae*, for the implementation of strategies for the control of mites on post harvest ornamental plants.

2. MATERIALS AND METHODS

2.1 Strains of Mites

Mites of the chlorfenapyr resistant strain were collected from a commercial chrysanthemum field in Campinas Municipality, State of São Paulo (SP), in 2009. Mites of the milbemectin resistant strain were obtained from a commercial cultivation of roses in Holambra County (SP), in 2010. The acaricide susceptible strain was collected from an organic cultivation of raspberry in Campos do Jordão County (SP) in 2010. After collection, the mite strains were reared continuously on bean plants, *Canavalia ensiformis* L., in laboratory conditions, at 25 ± 1 ºC, 70 ± 5% RH and a 14 h photoperiod.

2.2 Chemical Tests

Commercial formulated milbemectin (Milbeknock_ 5% EC, Iharabras S.A. Chemical Industries) and chlorfenapyr (Pirate, BASF S.A.) were used. Both chemicals were commercially available in the State of São Paulo. These tests were based on the method described by [13]. Twenty adult females of *T. urticae* were placed on a bean leaf disc (4 cm diameter) on water soaked cotton in a Petri dish (9 cm diameter). Prepared suspension of acaricide (2 ml) was sprayed onto the leaf disc mites using a Potter spray tower (Burkard Manufacturing, Uxbridge, UK), at 68.9 kPa. Preliminary tests indicated that 1.6 ± 0.1 mg/cm^2 of distilled water was sprayed on the leaf disc with this volume and pressure. Thereafter, the mites on the leaf disc were kept at 25 ± 1ºC and a 14 h photoperiod for 72 h (for milbemectin) or 48 h (for chlorfenapyr) after treatment. Individual mite survival was determined by touching each mite with a fine brush. Mites which were unable to walk at least a distance equivalent to their body length were considered dead. Only distilled water was applied for the control. Tests in which control mortality was equal or higher than 10% were not considered in this study. Each experiment was replicated at least three times. Pooled data were subjected to Probit analysis (POLO PC; LeOra Software 1987) and LC_{50} with respective 95% FL were estimated [9].

2.2.1 Selections for Resistance

Females from Campinas field strain were selected for resistance to chlorfenapyr under laboratory conditions from January to May 2010; and females from Holambra field strain were selected for resistance to milbemectin from December to April 2011. Fifty adult females on bean-leaf disc were sprayed with chlorfenapyr or milbemectin using the Potter spray tower, as described above. Increasing concentrations of chlorfenapyr and milbemectin were used for each selection. After five selections for acaricide resistance, the LC_{50} values reached 48 mg of a.i./l (active ingredient per liter) for chlorfenapyr, and 44 mg of a.i./l for milbemectin.

2.2.2 Selections for Susceptibility

The purpose of the selections for susceptibility was to remove the genes responsible for chlorfenapyr and milbemectin resistance, and then to produce strains more susceptible to the acaricides. The selections for chlorfenapyr and milbemectin susceptibility were conducted with *T. urticae* gravid females from Campos do Jordão field strain, from April to October 2010. The mites were placed individually on a bean leaf disc (2.5 cm diameter) on water-soaked cotton in a Petri dish for 48 h. Each female oviposited on average 13.2 eggs. After this period, the female was transferred to another leaf disc arena and treated with milbemectin using the Potter spray tower. Decreasing concentrations of chlorfenapyr and milbemectin were used for each selection. After Five selections for acaricide susceptibility, the LC_{50} values reached 0.067 mg of a.i./l (active ingredient per liter) for chlorfenapyr, and 2.21 mg of a.i./l for milbemectin.

2.3 Irradiation Tests

The irradiation tests were carried out at the Laboratory of Radiobiology and Environment, of the Center for Nuclear Energy in Agriculture of University of São Paulo (CENA/USP). Mites were irradiated in a Cobalt-60 source (Gammacell-220), at a dose rate of 0.424 Gy/minute.

2.3.1 Effect of Gama Irradiation on the Toxicity of Acaricides

Thirty adult females of milbemectin and chlorfenapyr selected strains (S and R) were placed on bean-leaf disc arenas and subjected to different gamma radiation doses (5, 10, 20, 40 and 80 Gy). Twenty four hours after the gamma radiation treatment, the mites were sprayed with 2 ml of acaricide suspension using the Potter spray tower. Different milbemectin concentrations were used for the resistant [62.47, 125, 250, 500, 1000 mg of a.i./l] and the susceptible [1.50, 3, 6, 12, 25 mg of a.i./l] strains of *T. urticae*. The concentrations of chlorfenapyr used for the resistant strain [3, 6, 12, 24 e 48 mg of a.i./l] were also higher than those for the susceptible strain [0.014, 0.028, 0.06, 0.12 e 0.24 mg of a.i./l]. In the case of the control, the mites were exposed to the same acaricide concentrations, but they were not exposed to gamma radiation. The mortalities were assessed 72 h after milbemectin treatment and 48 h after chlorfenapyr treatment. Pooled mortality data were subjected to Probit analysis [16] and LC_{10}, LC_{50} and LC_{90}, with respective 95% FL, were estimated.

2.3.2 Effect of Gama Irradiation on the Growth Rates of Acaricide Resistant and Susceptible Strains

Five females of each strain (Milbemectin R and S; Chlorfenapyr R and S) were irradiated with 100, 200 e 300 Gy. The females were kept in bean leaf disc arenas at 20 ± 5ºC and a 14h photoperiod. The total number of mites (eggs, larvae, protonymphs, deutonymphs and adults) was counted on the 10th day after the gamma radiation treatment to estimate the instantaneous growth rate for the different strains of *T. urticae*. The eggs were observed for an additional period of six days to verify their viability. Only viable eggs were considered for estimating growth rates.

The instantaneous growth rate (r_i) was calculated using the following formula:

$$r_i = \ln(N_f / N_o)/\Delta T$$

where Nf is the final number of animals, No is the initial number of animals, and ΔT is the number of days the experiment was run, solving a rate of population increase or decline for r_i yields. Positive values of r_i indicate a growing population, $r_i = 0$ indicates a stable population, and negative r_i values indicate a population in decline and headed toward extinction [22].

The number of eggs per female per day and the instantaneous rate of increase (r_i) for each strain (R and S) of *T. urticae* were compared using ANOVA and Tukey's test, for different gamma radiation doses; and *t*-test ($P < 0.05$) was used to compare the effect of gamma radiation on different strains of *T. urticae*. The experiment was replicated 5 times.

3. RESULTS AND DISCUSSION

3.1 Effect of Gama Irradiation on the Toxicity of Acaricides on the Mite Strains

The results indicated that mites exposed to doses of 5, 10, 20, 40 and 80 Gy presented similar susceptibility to milbemectin strain (Table 1) and chlorfenapyr strain (Table 2). The dose of 20 Gy was chosen due to the fact that the best concentration-mortality curves, for both acaricides, were obtained for this dose.

Table 1. Percentage of mortality of Milbemectin R and S strains of *Tetranychus urticae* exposed to gamma radiation (5, 10, 20, 40 and 80 Gy) and sprayed with 5 milbemectin concentrations (mg of a.i./l), (24 h after radiation treatment). Total number of mites used to obtain the curves (*n*).

Milbemectin R

Chemical	Radiation Doses (Gy)					
Doses	5	10	20	40	80	Control
n	150	150	150	150	150	150
1000	73.3	73.3	90	66.6	56.6	86
500	66.6	63.3	66.6	73.3	66.6	80
250	70	63.3	56.6	70	70	50
125	50	43.3	36.6	73.3	23.3	40
62.47	16.6	23.3	26.6	20	16.6	16.6

Milbemectin S

Chemical	Radiation Doses (Gy)					
Doses	5	10	20	40	80	Control
n	150	150	150	150	150	150
25	90	96.6	93.3	93.3	93.3	70
12	83,3	86.6	96.6	96.6	93.3	60
6	83,3	90	80	80	73.3	23.3
3	66,6	70	60	66.6	73,3	13.3
1.5	36,6	50	40	56.6	66,6	6.6

Table 2. Percentage of mortality of Chlorfenapyr R and S strains of *Tetranychus urticae* exposed to gamma radiation (5, 10, 20, 40 and 80 Gy) and sprayed with 5 chlorfenapyr concentrations (mg of a.i./l), (24 h after radiation treatment). Total number of mites used to obtain the curves (*n*).

Chlorfenapyr R

Chemical	Radiation Doses (Gy)					
Doses	5	10	20	40	80	Control
n	150	150	150	150	150	150
48	73.3	73.3	90	66.6	56.6	86
24	66.6	63.3	66.6	73.3	66.6	80
12	70	63.3	56.6	70	70	50
6	50	43.3	36.6	73.3	23.3	40
3	16.6	23.3	26.6	20	16.6	16.6

Chlorfenapyr S

Chemical	Radiation Doses (Gy)					
Doses	5	10	20	40	80	Control
n	150	150	150	150	150	150
0.24	90	96.6	93.3	93.3	93.3	70
0.12	83,3	86.6	96.6	96.6	93.3	60
0.06	83,3	90	80	80	73.3	23.3
0.028	66,6	70	60	66.6	73,3	13.3
0.014	36,6	50	40	56.6	66,6	6.6

Tests with the Milbemectin S strain showed an increased susceptibility to the acaricide milbemectin, when the mites were irradiated (20 Gy), in comparison to the control (non irradiated mites). The lethal concentrations of milbemectin for the irradiated mites were significantly lower than those of the control, based on the non overlapping of 95% confidential intervals of LC_{10}, LC_{50} and LC_{90} values. Considering the LC_{50} values, it was observed an increase of 5.5 times in the toxicity of milbemectin when the mites of the susceptible strain were exposed to gamma radiation (Table 3).

Some authors also observed an increase in mortality of insects [e.g. tropical warehouse moth (*Cadra cautella*); house fly] with the combined use of radiation and agrochemicals [8,6].

In the case of the Milbemectin R strain, there was no significant influence of gamma irradiation on the toxicity of milbemectin to the mites of this strain, with similar values of LC_{10}, LC_{50} and LC_{90}, in comparison with the control.

Table 3. Susceptibility of mites of milbemectin resistant and susceptible strains of *Tetranychus urticae* exposed to gamma radiation (20 Gy) and sprayed with milbemectin (24 h after radiation treatment). Total number of mites used to obtain the concentration-response curves (*n*); estimation of LC_{10}, LC_{50} and LC_{90} values (mg of a.i./l) (95% CL); slope and SE; Chi-square (χ^2) and probability; degree of freedom (df).

	Milbemectin			
	Resistant (R)		Susceptible (S)	
	20 Gy	Control	20 Gy	Control
LC_{10}	27.58 (8.27 - 51.02)	58.49 (30.91 - 86.02)	0.37 (0.112 - 0.80)	2.36 (1.182 - 3.54)
LC_{50}	183.49 (124.89 - 253.76)	234.51 (180.32 - 303.38)	2.15 (1.23 - 2.95)	11.88 (8.87 - 17.41)
LC_{90}	1219.78 (727.04 - 3264.35)	940.43 (646.34 - 1739.94)	10.86 (7.47 - 20.91)	58.92 (33.92 - 164.40)
n	150	150	150	150
Slope ± SE	1.559 ± 0.077	2.126 ± 0.095	1.830 ± 0.112	1.846 ± 0.094
χ^2	0.572	4.948	0.178	1.789
df	3	3	3	3
p	0.903	0.176	0.981	0.617

For the Chlorfenapyr S strain, the effect of gamma radiation was similar to that observed for Milbemectin S strain, with increased toxicity of chlorfenapyr to the mites of this susceptible strain. The chlorfenapyr LC_{10} and LC_{50} values were lower for irradiated mites than for non irradiated mites (Table 4).

In the case of Chlorfenapyr R strain, the mites exposed to gamma radiation showed to be more tolerant to chlorfenapyr, considering the LC_{10} values. The same trend was observed for the LC_{50} values, however, there was no significant difference with the control.

An increased tolerance to agrochemicals was also observed by [23] in *Musca domestica* L., when malathion was applied to flies previously exposed to gamma radiation. These authors suggest that increased tolerance to malathion in irradiated flies can be attributed to an increase of detoxifying enzymes.

Table 4. Susceptibility of mites of chlorfenapyr resistant and susceptible strains of *Tetranychus urticae* exposed to gamma radiation (20 Gy) and sprayed with chlorfenapyr (24 h after radiation treatment). Total number of mites used to obtain the concentration-response curves (*n*); estimation of LC_{10}, LC_{50} and LC_{90} values (mg of a.i./l) (95% CL); slope and SE; Chi-square (χ^2) and probability; degree of freedom (df).

	Chlorfenapyr			
	Resistant (R)		Susceptible (S)	
	20 Gy	Control	20 Gy	Control
LC_{10}	14.7 (6.23 - 21.69)	1.56 (0.051 - 3.99)	0.0096 (0.002 - 0.0105)	0.019 (0.017 - 0.029)
LC_{50}	78.99 (46.58 - 397.598)	48.37 (24.62 - 398.84)	0.038 (0.023 - 0.050)	0.067 (0.051 - 0.086)
LC_{90}	424.4 (145.07 - 17482.74)	1500.14 (242.364 - 1939532.70)	0.15 (0.106 - 0.295)	0.23 (0.17 - 0.37)
n	150	150	150	150
Slope ± SE	1.756 ± 0.253	0.860 ± 0.069	2.152 ± 0.192	2.410 ± 0.124
$\chi 2$	0.2	1.27	3.411	1.223
df	2	3	2	3
p	0.905	0.736	0.182	0.748

3.2 Effect of Gama Irradiation on the Growth Rates of Acaricide Resistant and Susceptible Strains

The experiments showed that doses of 200 and 300 Gy eliminated the mite populations of acaricide resistant and susceptible strains of *T. urticae*, in a period of ten days, causing 100% of mortality of eggs and active stages of the spider mite (Table 5). The dose of 100 Gy did not lead to total elimination of the mite population, but reduced significantly the egg viability in both strains. The highest effect was observed for the Chlorfenapyr S strain, for which the instantaneous growth rate (r_i = 0.102) of irradiated mites was 3.8 times lower than that of the control. The effect of gamma radiation was lower for the Chlorfenapyr R strain, with the r_i value 40% higher than that of the Chlorfenapyr S strain.

The experiment also showed that the highest gamma radiation doses affected significantly the oviposition rates. The lowest values in the number of eggs were observed for Milbemectin R and S strains of *T. urticae* exposed to 300 Gy.

Several authors have shown that insecticide resistant and susceptible strains of stored grain insects showed no significant differences in susceptibility to gamma radiation [2,4,21,17]. However, [7] showed that DDT-resistant strains of *Tribolium castaneum* (Herbst) were more radiosensitive as the wild-type strains. On the other hand, [18] noticed that a phosphine resistant strain of *T. castaneum* was more tolerant to radiation than a susceptible one.

Table 5. Effect of gamma radiation on the growth rates of acaricide resistant and susceptible strains of *Tetranychus urticae*. Mean estimated values (± SE) of number of eggs per female per day and instantaneous rate of increase (r_i) per day, for different radiation doses.

Strains	100 Gy		200 Gy		300 Gy		CONTROL	
	n eggs/female/day	r_i (day)	*n* eggs/female/day	r_i (day)	*n* eggs/female/day	r_i (day)	*n* eggs/female/day	r_i (day)
Milbemectin R	4.66 ± 0.35 a	0.148 ± 0.02	2.68 ± 0.80 ab	-[1]	1.86 ± 0.70 b	-[1]	4.74 ± 0.35 a	0.38 ± 0.01
Milbemectin S	4.32 ± 0.82 ab	0.148 ± 0.02	2.68 ± 0.87 ab	-[1]	2.28 ± 0.37 a	-[1]	5.12 ± 0.55 b	0.387 ± 0.01
Chlorfenapyr R	4.04 ± 0.32 ac*	0.143 ± 0.02*	2.36 ± 0.69 b	-[1]	3.84 ± 0.34 ab*	-[1]	5.48 ± 0.24 c	0.395 ± 0.01
Chlorfenapyr S	3.34 ± 0.86 ab*	0.102 ± 0.01*	2.40 ± 0.54 a	-[1]	2.92 ± 0.71 ab*	-[1]	5.12 ± 0.55 b	0.388 ± 0.01

Values followed by followed by a common letter, in the same row (and same parameter), do not differ significantly (Tukey's test, $P < 0.05$).

*Significant difference, between strains (R and S) (*t*- test; $P < 0.05$).

[1]Final number of mites equal zero.

4. CONCLUSIONS

Low gamma radiation doses (e.g.: 20 Gy) can be useful to increase the toxicity of the milbemectin and chlorfenapyr, especially in susceptible strains of *Tetranychus urticae*. However, mites of acaricide resistant strains of the spider mite (e.g.: Chlorfenapyr R Strain) can become even more tolerant to the agrochemicals (e.g.: chlorfenapyr) when previously exposed to gamma radiation.

Doses of 200 and 300 Gy eliminate the mite populations of acaricide resistant and susceptible strains of *T. urticae*, in a period of ten days. The dose of 100 Gy cannot lead to total elimination of the mite population, but reduce significantly the egg viability of *T. urticae*.

The development of acaricide resistance can affect the susceptibility to gamma radiation in *T. urticae*. This effect is observed for the Chlorfenapyr R strain, for which the population growth rate is less affected by the irradiation, in comparison with the susceptible strain.

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