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Effect of biofertilizers and Verticillium lecanii (Zimm.) Viégas applied to Canavalia ensiformis L. (Fabaceae) on the bioecology of Brevipalpus phoenicis (Acari: Tenuipalpidae)

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ABSTRACT

Two assays were conducted to evaluate the effect of biofertilizer suspensions applied to *Canavalia ensiformis* (L.) DC. plants on *Brevipalpus phoenicis* (Geijskes) bioecology. In the first assay (residual effect), the following treatments were tested: a) Distilled water (Control); b) Biofert - biofertilizer produced in a single bioreactor; c) Biomix – a mix of biofertilizers produced in four bioreactors; and d) Bio+VL - Biofert + *Verticillium lecaniiI* (Zimm.) Viégas. In the second assay (systemic effect) only two suspensions were tested: a) Biofert and b) Distilled water (control). In the first assay, the biofertilizer was applied once on the whole plant and mites were confined in arenas on both leaflets. In the second assay, the plants were sprayed weekly only in one leaflet and the mites were confined in arenas on the non-sprayed leaflet. In both bioassays, bioecological parameters related to survival and oviposition were evaluated. The biofertilizer effects on potential population growth during the first generation were measured by fertility life table parameters (net reproductive rate (Ro), mean generation time (T), doubling time (DT), intrinsic rate of increase (Rm) and finite rate of increase (λ). The biofertilizer had adverse effects on both survival and oviposition parameters. The net reproduction rates (Ro) were of 18.1; 12.9; 12.5 and 10.5 females/female (assay I) and 19.4 and 13.0 females/female (assay II), respectively for the treatments in the above-mentioned orders. These

results show that the biofertilizer reduced the potential population growth of *B. phoenicis* in either residual or systemic effect assays.
Key Words*Brevipalpus phoenicis*, biofertilizer, fertility life table, bioecology, fecundity, longevity..

INTRODUCTION

IN THE BRAZILIAN CITRUS groves, the management of the mite *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) is based on application of chemical pesticides. This practice promotes mite development of the resistance and the elimination of natural enemies. *B. phoenicis* is a cosmopolitan and poliphagous pest, vector of some Rhabdovirus, etiological agents of diseases that damage several crops (Childers et al., 2001). The cost of mite control represents 52% of the total cost of citrus production and corresponds to 90% of the total acaricides marketed in Brazil for all crops (Omoto, 1998; Neves, 2000).

Biofertilizers produced throughfermentation of organic compounds enriched with mineral nutrients have been a low-cost and ecological alternative for pest and disease management (Santos and Akiba, 1996; Tratch and Bettiol, 1997). Their mode of action is related to phenomena such as induced systemic resistance, antibiosis or trophobiosis (Chaboussou, 1982; Chaboussou, 1985; Pinheiro and Barreto, 1996).

The objective of this research was to evaluate the residual and systemic effects of biofertilizers applied in Jack bean plants (*Canavalia ensiformis* L.) (Fabaceae) on the bioecology *B. phoenicis*. This was accomplished by using fertility life tables.

Fertility life tables have been used for evaluation of chronic effects of chemical and biological pesticides on the bioecology of target and non-target organisms (Nardo et al., 2001, Nascimento et al, 1998). That method integrates fertility and survivorship parameters allowing a more holistic approach of the treatment effects on mite population (Coppel and Mertins, 1977).

MATERIAL AND METHODS

Mite rearing.Miteswere collected from an area free of chemical acaricide applications, reared in adhesive Tanglefoot® arenas, on *Citrus sinensis* (L.) paraffinic fruit. The mite population was maintained in laboratory at 25±3 ºC, 70±10% RH and a 14:10 L:D photoperiod.

Biofertilizer.The biofertilizer was produced in a open at 26 ± 5ºC and 70 ± 10% RH, using a composting process in liquid media, comprising aerobic and anaerobic microbial digestion and fermentation. The bioreactors were plastic containers with 80 cm in diameter and capacity of 100 liters. The initial composition of the mixture was: 20 L of bovine manure, 10 L of bovine rumen content, 5 kg of enriched organic compound (Microgeo® , Microbiol, Limeira, SP, Brasil) plus an amount of chlorine free water to achieve 100 L of mixture. The mixture was shaken twice a day to promote aeration, gas elimination and equilibrium of the microbial community. After 35 days of biodigestion, samples with pH 6.5 were taken for evaluation in both bioassays.

Residual-effect bioassay. Plants of *C. ensiformis* in the leaflet stage were used as indicators of the biofertilizer action. The biofertilizer, dissolved to 10% in distilled water, was used in the following treatments: a) distilled water (control); b) Biofert – biofertilizer derived from only one bioreactor; c) Biomix – mixture of equal parts from four different reactors; and d) Bio+VL – biofertilizer + *Verticillium lecanii* fungus (isolate 972-ESALQ), at 3 x 10^5 conidia/mL.

The assays were conducted in environmental chambers at 25±3 ºC, 70±5% RH and 14:10 L:D photoperiod. The suspensions were applied once in each experimental unit (10 plants) with application rate of 20 mL per plant and pressure of 5 pound $inch^{-2}$. One 3.0-cm diameter adhesive Tanglefoot® arena was constructed in each leaflet. The residual effect of biofertilizers was evaluated on adult females and their offspring:

Evaluation of residual effect on adult females. Each arena was infested, on average, with 15 adult females of *B. phoenicis*, exposed to the treatments per five days. At the end of this period the total number of eggs per female and the number of living females were record in order to evaluate treatment effect on adult female survivorship and fecundity.

Evaluation of residual effect on offspring. After the removal of eggs produced during the initial 5 days, the females were kept on the plants per 24 additional hours to allow oviposition. After the adult emergence, the daily number of living female and the daily number of eggs laid per female in each treatment were recorded.

Systemic-effect bioassay. Only two treatments were tested: 1) Biofertilizer at 5% in distilled water (Biofert) and 2) distilled water (control). The suspensions were applied weekly only on one leaflet in each et 10 plants per treatment. Tanglefoot arenas were constructed as described above on the non-sprayed leaflets. Each arena was infested, on average, with 15 adult females of the mite *B. phoenicis*, maintained on the leaflets during 24 hours to allow oviposition. After that, the plants were transferred to laboratory conditions at 25±3ºC, 70±10% RH and 14:10 L:D photoperiod. In this assay, no daily evaluations were made in order to avoid stressing plants with excessive manipulation. They were made only at 28, 37, 41, 45 and 55 days after the beginning of oviposition. In consequence, it was not possible to evaluate egg viability and immature stage survivorship. The biological parameters for the adult stage

were estimated using the same procedures of the previous assay.

Statistical analysis. Survival and fertility data were used to estimate the following associated life table parameters: net reproductive rate (Ro), mean generation time (T), doubling time (DT), intrinsic rate of increase (Rm) and finite rate of increase (λ) (Southwood, 1978). Also, the bioecological parameters, mean egg viability, immature stage survivorship, means numbers of eggs laid per female, and mean adult longevity, were also estimated.

The life table parameters and their respective standard errors were estimated by jackknife method (Meyer et al., 1986) and the treatment means compared by t-tests using the jackknife standard errors. These analyses were performed using the software LIFETABLE.SAS (Maia et al. 2000).

The mean daily numbers of eggs laid per female were compared by F-tests for contrasts (Montgomery, 2000), after performing one-way analysis of variance using the ANOVA procedure (SAS Institute, 1998). Linear models fitted by weighted least squares (Grizzle et al., 1969) were employed to evaluate the treatment effects on egg viability and immature stage mortality as described in Forthofer and Lehnen (1981). They were modeled as binomial random variables and the treatment means were compared by Wald tests (Wald, 1943) using the CATMOD procedure (SAS Institute, 1998).

Curves describing the adult emergence and survivorship patterns across time in each treatment were estimated by Kaplan-Meier method (1958). Emergence and survival curves were compared by Log-Rank tests (Mantel, 1966) using LIFETEST procedure (SAS Institute, 1998).

The methods above were used in both bioassays except for adult survivorship. In the second bioassay, the survival functions were estimated by generalized linear models applied to interval censor data (Allison, 1997) instead the Kaplan-Meier method. This was done because the exact day of death is not known but only the time intervals were it occurred. The survival functions were compared by likelihood ratio tests. This analysis was performed using the GENMOD procedure (SAS Institute, 1998).

RESULTADS AND DISCUSSION

ResultsResidual-effect bioassay. Evaluation of residual effect on adult females. No adult female mortality was observed during the first five days of feeding on *C. ensiformis* plants. The oviposition rates in the treatments Biofert and Bio+VL were significantly lower than the one observed in the control (F-test for contrasts; $P < 0.05$) (Table 1).

Table 1. Residual effect of biofertilizers on the oviposition of *B. phoenicis* reared on *C. ensiformis* plants for five days after application of biofertilizers.

Treatment	females	Daily number eggs/female	SEM
Control	124	0.71[a]	0.05
Biomix	145	0.61[a]	0.06
Bio+VL	125	0.44b	0.05
Biofert	131	0.22c	0.06

Table 1.[a] Means within a column followed by the same letters are not significantly different (F-test for contrasts; $P< 0.05$).

Evaluation of residual effect on offspring. The egg viability was significantly affected by treatments with biofertilizer (Table 2): reductions of 14. 20 and 27% were observed for the treatments Biomix, Biofert and Bio+VL, respectively, with respect to the control. The median time to achieve adulthood was little affected by biofertilizers; it varied from 24 to 26 days, with low variability among individuals within treatments.

Table 2 – Residual effect of biofertilizer on viability of *B. phoenicis* eggs originated from females reared on *C. ensiformis* plants during five days.

Treatment	eggs	Viability (%) [a]	SEM (%)	*P*-values [b] Control	Biomix	Biofert
Control	119	89.10a	2.85	-	-	-
Biomix	102	76.69b	4.16	0.0143	-	-

Biofert	108	70.80bc	4.20	0.0004	0.3229	-
Bio+VL	122	64.70c	4.73	<0.0001	0.0571	0.3397[a]

Table 2.[a] Means within a column followed by the same letters are not significantly different (Wald test; $P < 0.05$).
[b] Probability of type I error associated to Wald tests for contrasts between each pair of treatment means.

All the treatments differed from control with respect to there the patterns of adult emergence across time ($P < 0.0109$, Log-rank test). Mites in Biomix and Bio + VL showed a similar pattern of emergence (Fig. 1) and got adult stage earlier than the ones in the control, while whose in Biofert got to this stage later than the control. Biomix and Bio+VL The treatments with biofertilizer decreased significantly ($P < 0.05$, Wald test) the mite survival, causing adjusted mortality (Abbott, 1925) of 12.4, 15.9 and 35.3%, respectively, for Biofert, Biomix and Bio + VL (Table 3).

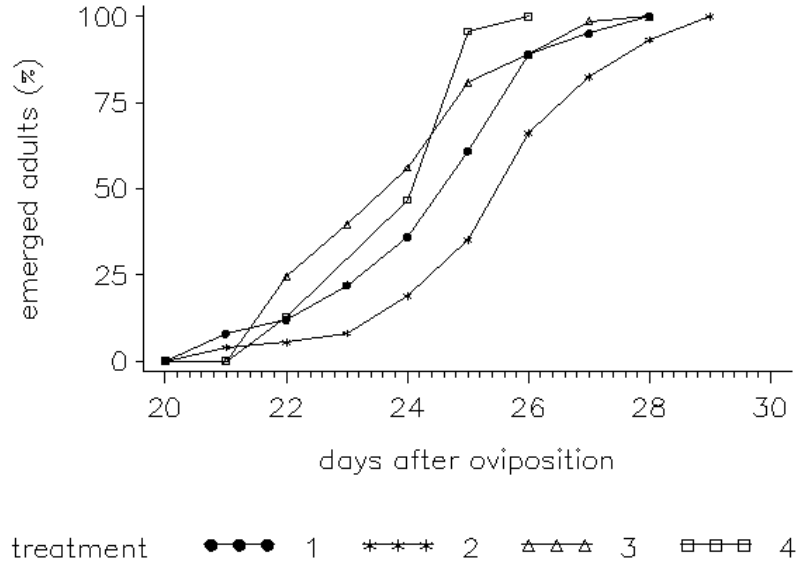

Fig. 1. Emergence patterns across time of *B. phoenicis* reared on *C. ensiformis* plants submitted to the biofertilizer treatments: 1 – Control, 2- Biofer, 3- Biomix and 4 – Biofert + VL. (Bio + VL).

Table 3. Residual effect of biofertilizers on the immature stage mortality of *B. phoenicis* reared on *C. Ensiformis* plants.

Treatment	eggs	Emerged adults	Mortality (%) ± SEM	Adjusted mortality Abbott (1925) (%)	*P*-values [a]		
Control	97	86	11.4 ± 3.40	-	-	-	-
Biofert	94	74	22.3 ± 4.29	12.4	0.0405		
Biomix	102	76	25.5 ±4.31	15.9	0.0086	0.6050	
Bio+VL	82	47	42.7 ± 5.46	35.3	< 0.0001	0.0034	0.0135

Table 3.[a] P-values associated to Wald tests for contrasts between pairs of treatment means.

The fecundity of adult female offspring's in biofertilizer treatments averaged 26.2 eggs/female and slightly lower than the one observed in control (32.8 eggs/female) (F test; $P = 0.11$) (Table 4). The mortality patterns across time were similar in all treatments (Fig. 2), indicating a lack et effect of biofertilizers on adult offspring longevity (Log-rank test; $P > 0.13$).

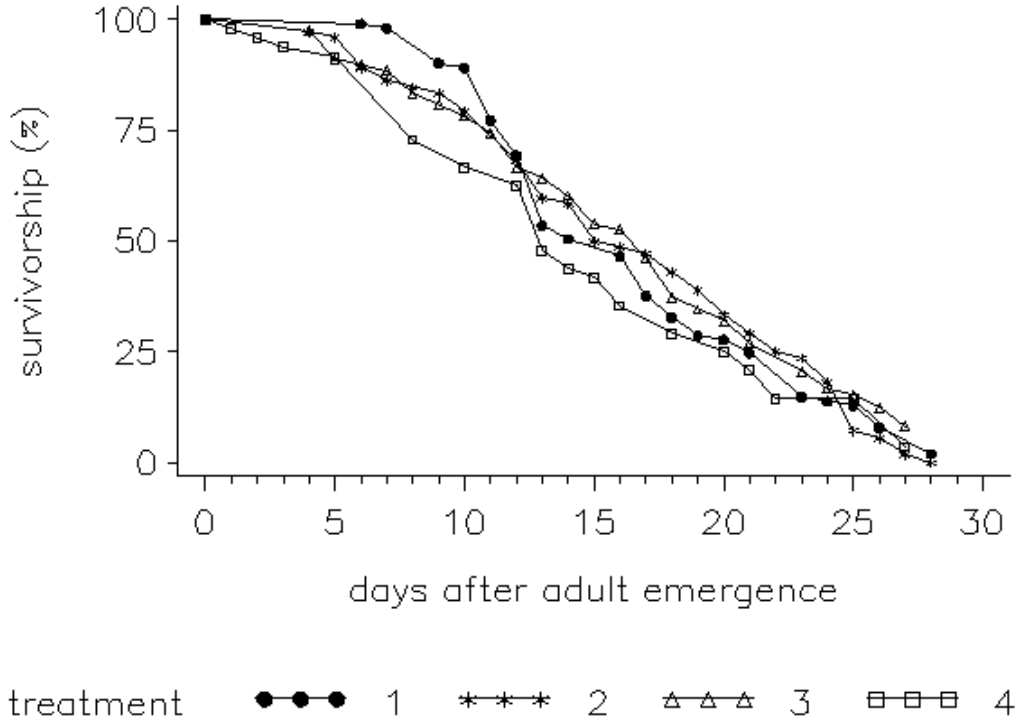

Fig. 2. Survival curves of adult *B. phoenicis* reared on *C. ensiformis* plants submitted to the following treatments: 1 – Control, 2 – Biofertilizer (Biofert), 3 – Biomix and 4 – Biofertilizer + *V. lecanii* (Bio + VL).

Table 4. Residual effect of biofertilizers on mean number of *B. phoenicis* eggs laid per femalereared on *C. Ensiformis* plants.

Treatment	eggs/female	SEM
Control	32.81	2.34
Biofert	25.52	2.34
Biomix	27.48	2.09
Bio+VL	25.48	2.34

Estimates of Ro values showed that the mite population increased 180 X (Table 5), in one generation in the control, whereas in the Biofert, Biomix and Bio + VL treatments the net reproductive rates were lower: 12.9, 12.5 and 10.5 female/female, respectively (Jackknife t test; $P < 0.01$). The mean generation time (T) in the Biofert and Biomix treatments were significantly higher than those ones in the control (Jackknife t-test; $P < 0.003$), indicating that for a fixed time interval, the number of *B. phoenicis* generations is greater in the control.

Table 5. Estimates of associated fertility life table parameters with respective standard errors and *P*- values corresponding to Jackknife t-tests for comparison between pair of treatment means.

Parameter	Treatment	Estimate ± SEM	P-values		
			Control	Biofert	Biomix
Ro (female/female)	Control	18.09 ± 0.92	-	-	-
	Biofert	12.91 ± 0.89	0.0006[b]	-	-
	Biomix	12.53 ± 0.57	0.0001	0.3609[b]	-
	Bio+VL	10.52 ± 0.65	0.0001	0.0252	0.0177[b]
T (days)	Control	25.47 ± 0.40	-	-	-
	Biofert	28.16 ± 0.43	0.0002[a]	-	-
	Biomix	27.30 ± 0.44	0.0038	0.0936[a]	-
	Bio+VL	26.27 ± 0.42	0.0980	0.0038	0.0557[a]
DT (days)	Control	6.09 ± 0.13	-	-	-
	Biofert	7.61 ± 0.32	0.0008[a]	-	-
	Biomix	7.48 ± 0.16	0.0001	0.6386[a]	-
	Bio+VL	7.72 ± 0.14	< 0.0001	0.3756	0.1420[a]
Rm	Control	0.1137 ± 0.0025			
	Biofert	0.0909 ± 0.0038	0.0001		
	Biomix	0.0926 ± 0.0020	< 0.0001	0.3493[a]	
	Bio+VL	0.0897 ± 0.0017	< 0.0001	0.3870[b]	0.1450[b]
λ	Control	1.1204 ± 0.0028			
	Biofert	1.0951 ± 0.0041	0.0001		
	Biomix	1.0970 ± 0.0022	< 0.0001	0.3488[a]	
	Bio+VL	1.0938 ± 0.0018	< 0.0001	0.3876[b]	0.1451[b]

The intrinsic rate of increase (Rm) estimates were positive in all treatments, showing that population was increasing during the period of evaluation. The λ values, the constants by which the population is expected to multiply each day, were 1.120, 1.0951, 1.097 and 1.093 in the control, Biofert, Biomix and Bio + VL treatments respectively. The showing daily rate of increase was higher in the control (12.04 % per day) when compared to that rate in the biofertilizer treatments, that averaged 9.51% (Jackknife t-test, $P < 0.01$).

There was no difference between Biofert and Biomix treatments regarding life table parameters (Jackknife t-test; $P <$ 0.3388). That is, the mixture of biofertilizers produced in different reactors (Biomix) kept the same tendency of interaction with the plant and the same deleterious effect on the mite growth potential than the one of the biofertilizer from just one container (Biofert).**Systemic effect of biofertilizer**. The immature stage mortality of *B. phoenicis*) were 28% and 39% in the control and sprayed plants, respectively. The adjusted mortality of mites reared on sprayed plants was 15.27% higher than control (Wald test, $P = 0.0707$). The mean number of eggs laid per female in sprayed and non-sprayed plants did not differ between them and averaged 35.35 eggs/female (F test; $P =$ 0.9136), showing no systemic effect of biofertilizer on fecundity.

The survival patterns of adult female *B. phoenicis* in the sprayed and non-sprayed treatments were significantly different (Log-Rank test, $P< 0.001$) The biofertilizer treatment caused a reduction of 3.6 days in the median mite longevity, corresponding to 17% of the longevity in the control (21.3 days. Mites in the biofertilizer treatment completed the reproductive stage earlier Fig. 3), contrary to the observed in the residual effect assay, were the survival curves were not affected by the treatments. This result shows that the systemic action of biofertilizers implied on reduction of female longevity and consequently on the duration of oviposition period and generation time.

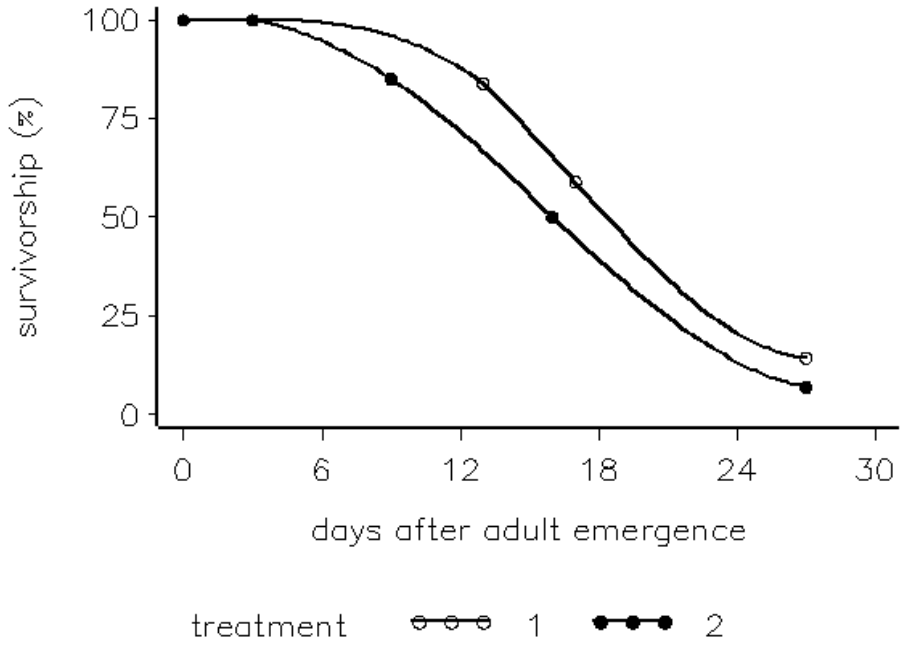

Fig. 3. Survival patterns of adult female *B. phoenicis* reared on non-sprayed (Treatment = 1) and sprayed plants (Treatment = 2) (Log rank test, $P< 0{,}0001$).

The Ro estimates showed that mite population increased 19 times in non-sprayed plants (control) and 13 times in those ones sprayed with biofertilizer (Table 6). The mean generation time (T) was two days shorter for mites reared on sprayed plants and consequently, the doubling time was longer. The Rm values were positive for both treatments, indicating an increase in the mite population. The estimates of finite rates of increase (λ), derived from Rm values showed 8.72% and 7.96% daily rates of increase in the mite populations in control and biofertilizer treatments, respectively. These results demonstrate a deleterious systemic effect of the biofertilizer on the mite population growth potential.

Table 6. Estimates of associated fertility life table parameters of *B. phoenicis* with respective standard errors and *P*-values corresponding to Jackknife t-tests used to compare treatment means.

Parameter	Treatment	Ro ± SEM (female/female)	*P*-values
Ro (female/female)	Control	19.42±0.87	
	Biofert	13.00±1.35	< 0.0001 a
T (days)	Control	35.46±0.10	
	Biofert	33.46±0.23	<0.0001 a
TD (days)	Control	8.28±0.13	
	Biofert	9.00±0.38	0.0520 b
R_m	Control	0.0837±0.001	
	Biofert	0.0768±0.003	0.0368 a
λ	Control	1.0872±0.001	
	Biofert	1.0796±0.003	0.0363 a

Discussion

The biofertilizer acted as an oviposition inhibitor when adult females were reared on sprayed plants, in both residual and residual assays. This is a promising result, if such a deleterious effect is reproducible under field conditions. Considering the crops where *B. phoenicis* occurs, this kind of action may result in significant population suppression.. In the residual effect assay, no *V. lecanii* infection symptoms were observed on adult mite females during the 5-day exposure. This microbial agent, despite being considered epizootic and being used in commercial scale in mite control (Faria and Magalhões, 2001), was not infective in the tested concentration. The colonization of *V. lecanii* in *B. phoenicis* mite occurs between 72 and 120 hours after inoculation and mortality occurs after that period (Macedo et al., 2001). This fungus has low reproductive capacity and is very dependent on temperature and humidity. It is possible that biofertilizer compounds had deleterious effect on the fungus.

Because adult mites had suffered some kind of physiological injury during the reproductive stage, it was necessary to evaluate the negative results on offspring growth and development. The indirect effects evaluated on offspring individuals showed a reduction in egg viability in all treatments. This result suggests a combined action of biofertilizer compounds and/or a response of plant systemic defense that affected the mite physiology and consequently the egg viability. Deleterious effects on fecundity also were observed in *Tetranychus urticae*, on the same host plant (Medeiros et al. 2000 and 2001).

Plant stresses cause alterations in its physiological and adaptive patterns, inducing cellular reactions. The mechanisms of plant-herbivore interaction are little known, however, in the interaction biofertilizer-plant, similar reactions involved in plant-pathogen interrelation may have occurred. Changes induced by stress caused by microbial agents, pathogenic or non-pathogenic, have been reported as mechanisms of plant defense (Dempsey et al., 1998; Gatehouse and Gatehouse, 1998).

Due to amplitude of biotic and abiotic compounds in its composition, the biofertilizers, besides acting as nutritional factor, may be considered potential elicitors of induced resistance mechanisms in plants. The probability of simultaneous activation of different active sites is high, considering the plant and biofertilizer complexities. Such action results in expression of various genes that enables the induction or amplification of systemic resistance mechanisms, independent of presence of herbivorous in the plant. (Azevedo et al., 2000; Silva Filho et al., 2000).

There was no significant effect of biofertilizer on mite offspring fecundity. Considering that biofertilizer had been completely metabolized during the period between application

and offspring oviposition, it is possible to infer that in the absence of elicitor agent the defensive plant response disappeared (Cordeiro and Sá, 1999). The biofertilizer pulverization must be done in the beginning of infestation and repeated, initially, at short intervals of time (days), to reach individuals of different generations and different reproductive ages. This strategy may reduce rapidly the population growth and must be continued until the population gets to a desirable level. From this point on, the biofertilizer can be applied at longer intervals (months), keeping the trophobiotic equilibrium between the crop and phytophagous mite.

A similar study using chemical fertilizers (Sudoi, 1993) showed that biochemical factors may influence the defense mechanism of plant. Some nutrients in tea plant (*Camellia sinensis*) (zinc sulfate, zinc oxide, urea and ammonia sulfate) can affect the *B. phoenicis* oviposition. Losses in tea yield caused by this mite have been with to excessive plant nutrition with inorganic nitrogen. High doses of inorganic nitrogen in soil increased the *B. phoenicis* population and also the symptoms of foliar limb necrosis provoked by this mite in tea plants, in Kenya. However, application between 100 and 200 kg of N ha^{-1} induced plant tolerance and reduced the losses caused by the mite, without affecting the crop yield (Sudoi et al., 2001).

Wermelinger et al. (1985) studying the effect of N fertilization on *Tetranychus urticae* verified that increasing contents of N, reduced the plant phenolic compounds and increased fecundity, oviposition rate and dry weight of the mites. The amino acid levels, sugars and potassium in plant were increased They also observed negative correlation between level of phenolic compounds and population growth parameters (Ro, Rm and fecundity) and positive correlation only between that levels and mean generation duration (T).

Based on estimates of fertility life table parameters, it is possible to assert that in the plants treated with biofertilizer in the presence or absence of the fungus *V. lecanii* the *B. phoenicis* population dynamics was severely affected, with reduction in its population growth potential. These results suggest that a systemic effect in plants cause severe damage to the mite population. However, it is necessary to conduct biochemical studies to elucidate the expression of new compounds in plants and changes in the concentration of existing compounds. This information will certainly to improve the understanding of biofertilizer mode of actions and contribute to a more efficient management of *B. phoenicis* population.

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