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Interactions between Host Plants and Biofertilizers: Contact Effect on Biological Parameters of the Mite Brevipalpus phoenicis

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ABSTRACT

This study was conducted aiming to evaluate interactions between deleterious action of biofertilizers on *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) and host plants. In the first bioassay the following concentrations were tested: 0% (control), 5%, 15%, 30% and 50%. The experimental unit was a leaf of *Ligustrum lucidum* plant (six leaves per treatment). Twelve mites were liberated in arenas on each leaf. The second bioassay was run on 20 days old *Canavalia ensiformis* plants, using ten plants per treatment. Ten mites were transferred for arenas on each host plant cotyledonal leaf. Five concentrations were also tested: 0%, 5%; 10%; 20% and 30%. The adult female mortality and the number of eggs laid in each arena were quantified daily during 72 h in the first assay and 120 h in the second one. The mite survivorship and oviposition were significantly reduced with the increase of biofertilizer concentration in both experiments. The LC25 estimates for the 24, 48 and 72 h periods were respectively 50,04; 15,70 and 4,95% in *L. lucidum*. The LC_{25} and LC_{50} estimates for the 24, 48, 72,96 and 120 h periods in *C. ensiformis* were 8,15; 7,78; 0,63; 0,68 and 0,63 and of 19,64; 19,03; 2,38; 2,60 and 2,67, respectively. No LC50 estimates were obtained in *L. lucidum*, due the low mortality rates. The biofertilizer had deleterious action on fertility and survival of *B. phoenicis* on both host plants, being more severe on *C. ensiformis*. The mites dead by the biofertilizer action showed evidences of microbial colonization. A colloidal compound of the biofertilizer induced mite immobilization and obstruction in its digestive tract.

Key Words*Brevipalpus phoenicis*, biofertilizer, fertility life table, bioecology, fecundity, longevity..

INTRODUCTION

Brevipalpus phoenicis (Geijsks) (Acari: Tenuipalpidae) is one of the most common mite worldwide, primarily throughout the tropical regions (KENNEDy et al. 1996). It is a poliphagous cosmopolitan mite, vector of Rhabdovirus, principal pest in the Brazilian citrus groves (OMOTO 1998). The control of that mite, made with chemical products has promoted evolution of resistant strains and reduction of the natural enemies' populations. Besides, the cost of mite control represents 52% of the total citrus production cost in the São Paulo state, Brazil, and corresponds to 90% of the total amount of acaricides marketed in Brazil for all crops (OMOTO 1998, NEVES 2000).

The use of organic products that act as fertilizers and plant protectors, mainly liquid biofertilizers, is growing in the Brazilian organic agriculture. Biofertilizers have been a low cost and ecological alternative for pest and disease management (SANTOS AND AKIBA 1996, TRATCH AND BETTIOL 1997). They are produced through biodigestion of organic compounds enriched with mineral nutrients resulting in a mix of bioactive compounds (microorganisms, metabolites and mineral chelates). Due to its dynamic molecular composition, the biofertilizers possess nutritional activity on the plant and acting by trophobiosis, they also work as regulators and stimulants of plant defense responses (CHABOUSSOU 1982, PINHEIRO AND BARRETO 1996, POLITO 2001, D'ANDRÉA 2001). Their principles of action are related to phenomena such as induced systemic resistance, antibiosis and trophobiosis (CHABOUSSOU 1985, PINHEIRO AND BARRETO 1996). Fungistatic, antibiotic and insecticidal action of biofertilizers has been also reported (SANTOS AND AKIBA 1996, TRATCH AND BETTIOL 1997). Some harmful effects of biofertilizers on mite populations have been observed in several vegetable crops (SANTOS 1991, CUNHA ET AL. 2000, NUNES AND LEAL 2001), coffee and citrus (ALVES et al. 2001). However, the nature of interaction between host plants and biofertilizers effect on phytophagous mites needs to be better understood. Laboratory studies indicate that biofertilizers interfered on the *B. phoenicis* and *Tetranychus urticae* oviposition (MEDEIROS et al. 2000a, MEDEIROS et al. 2000b, MEDEIROs et al. 2001). However, those effects are not usually considered in studies of biological or chemical control.

The objective of this research was to evaluate interactions between host plant and contact action of biofertilizers on *B. phoenicis*, quantifying the effect of increasing biofertilizer concentrations on survival and fertility of mites reared on *Ligustrum lucidum* (L.) (Oleaceae) and *Canavalia ensiformis* (L.) DC. (Fabaceae) plants.

MATERIAL AND METHODS

Mite rearing– mites were 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°C, 70±10% RH and a 14:10 L:D photoperiod.

Biofertilizer – The biofertilizer was produced in an open at 26 ± 5°C 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 COM – Microbiol Ind. And Com. Ltda.) 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.

Contact action on mites reared on *Ligustrum lucidum* leaves.

The bioassay was accomplished to quantify the effect of increasing biofertilizer concentrations on mite survival and fecundity, using a completely randomised design. The mites were reared on young *L. lucidum* leaves, in Petri dishes (15 cm diameter). The leaves were washed with water and put in the Petri dishes (2 leaves/dish) with the abaxial surface turned upwards, on polyurethane foam (0.8 cm thickness), soaked with distilled water. The leaves were circled with a thin moistened cotton layer in order to keep the leaves turgid. Arenas (3 cm diameter), delimited with Tanglegfoot®, were made on each leaf.

The treatments were five concentrations of the biofertilizer, diluted in distilled water: 0% (control), 5%; 15%; 30% and 50%. The experimental unit was a leaf of *L. lucidum* plant (six leaves per treatment). Twelve mites were liberated in arenas on each leaf. They were sprayed (5-pound pol^{-1}) with 2 mL of the biofertilizer suspension and maintained in laboratory (26±0.5°C, 70% R.H. and 14:10 L:D photoperiod).

The number of living adult females and the number of eggs laid in each arena were quantified daily during 72 h. These data were used to calculate mortality and mean daily number of eggs laid per female.

To describe the effect of biofertilizer concentration on mite mortality, the following dose-response models were fitted for each evaluation date:

$$Y_{ij} = \beta_0 + \beta_1 . (log (x_i + 1)) + \varepsilon_{ij}$$

where Y_{ij} is the logit of the observed mortality (p_{ij}) at concentration i and arena j; β_0 is the intercept, β_1 the slope, x_i the concentration i and ε_{ij} the random error associated to Y_{ij}.

The logistic models were fitted using the GENMOD Procedure of the SAS® System (SAS Institute 1998). The statistical significance of parameters was evaluated by likelihood tests (Forthofer and Lehnen 1981). Based on the fitted models, lethal concentrations were calculated for each evaluation date.

A negative exponential model was adjusted to evaluate the concentration effects on mite oviposition. This model is described above, in its linearized form:

$$Ln (Y_{ij}) = \beta_0 + \beta_1 . x_i + \varepsilon_{ij}$$

where *Yij* is the daily number of eggs laid per female for the concentration *i* and arena *j*; β_0 is the model intercept, β_1 the slope, x_i the biofertilizer concentration *i* and εij the random error associated to *Yij*.

The parameters were estimated by ordinary least squares method using the REG Procedure of the SAS System (SAS Institute 1998). One-tailed t-tests were performed to evaluate the significance of model parameters (MONTGOMERY 2000).

Contact action on mites reared on *Canavalia ensiformis*.

The assay was made on 20-days-old *C. ensiformis* plants cultivated in 300 ml plastic pots containing Plantemax® substratum. The experimental design was completely randomised, with 10 plants per treatment. Ten mites were transferred for circular Tanglegfoot® arenas (3.0 cm diameter) constructed on the adaxial surface of the cotyledonal leaves.

The topical and residual action of the biofertilizer was evaluated on survival and fecundity of the mite adult females. Five concentrations of the biofertilizer, diluted in distilled water, were used: 0% (control), 5%; 10%; 20% and 30%. The plants were sprayed with the biofertilizer suspension (10ml per plant) and maintained at laboratory (26±3°C; 70±5% R.H. 14:10 L:D photoperiod).

The female mortality and the number of eggs laid in each arena were quantified daily during 120 h. At the end of each observation the eggs were removed from the arenas.

For the evaluation times 24 and 48 h, the models used to describe the effect of biofertilizer concentration on mite mortality were the same ones adopted in the previous bioassay. For the

other evaluation times (72, 96 and 120 h), the following non-linear models were adjusted:

$$p_{ij} = \beta_0 + \beta_1.(1 - exp(-\beta_2.x_i)) + \varepsilon_{ij}$$

where *pij* is the proportion of death mites, corresponding to the concentration *i* in the arena j; β_0 is the intercept of the model, that corresponds to the proportion of deaths in the control, β_1 the difference among the mortality in the control and the maximum mortality, β_2 is a parameter related to the increment of the mortality in function of the concentration *xi,* and *eij* the random error associated to each observation.

The parameters were estimated by non-linear least squares method using the NLIN Procedure of the SAS System (SAS Institute 1998). The significance of the parameters was evaluated through 95% asymptotic confidence intervals. The effect of concentration on oviposition was evaluated using the same methods described in the previous bioassay.

Pathological action of the biofertilizer on *B. phoenicis.*

The behaviour and the death of the mites by the pathological action of the biofertilizer were characterized. The pathological alterations were evaluated by scanning electronic microscopy (SEM). Samples of dead mites were collected 24 hours after the death in leaves of *C. ensiformis*, with and without biofertilizer. The image analysis was made at the "Núcleo de Microscopia Eletrônica Aplicada à Pesquisa Agrícola, NAP/MEPA". The copies were disposed in metallic supports ("stubs"), adhered by double-face polycarbonate ribbon and fixed during a 48 hours period in steam of osmium tetroxide (OsO4) to 2%. The stubs were exposed to osmium steams in a camera covered with an aluminium film. Later, the samples were maintained in glass dryer with silica per 48 h. Before the observation in SEM, the material was metallized with gold, for 180s, in a Balzers evaporator, MED-010. The photomicrographs were generated in high vacuous SEM (LEO 435 VP) with 500 to 10,500 X magnifications and 20 kV.

RESULTADS AND DISCUSSION

Contact action on mites reared on *Ligustrum lucidum* leaves.

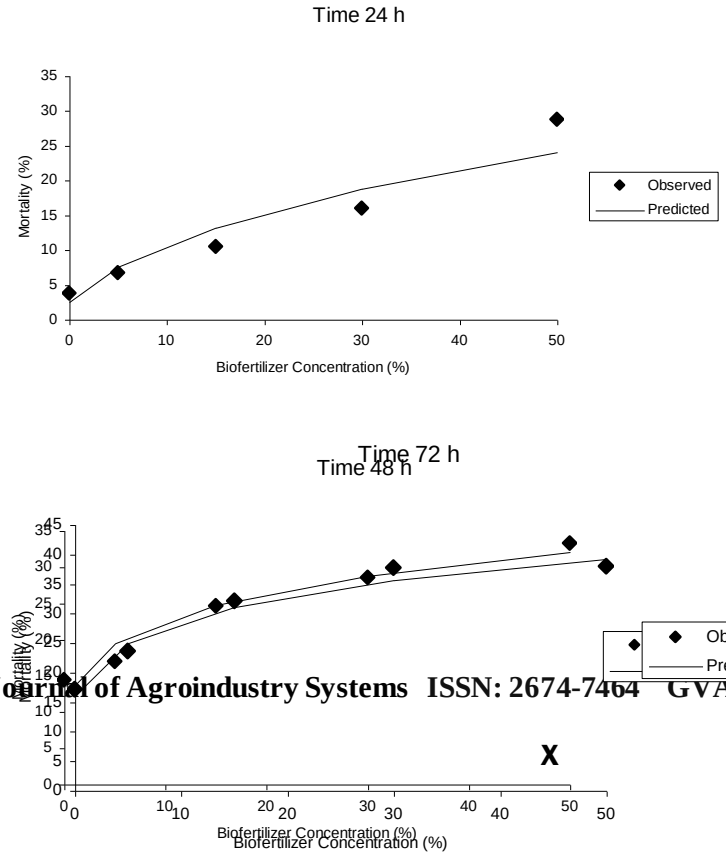
The mite survivorship was significantly reduced with the increase of biofertilizer concentration ($p < 0,01$) in all evaluation times. The proposed logistic models showed adequate goodness-of-fit with R^2 values of 97; 98 and 99% for 24, 48 and 72 h, respectively (Table 1). The mite mortality increased with the increase of the biofertilizer concentration. This increase was fast, in the low concentrations and slow in the high concentrations (Fig. 1).

Table 1. * P-values associated to likelihood ratio tests (x^2).
Table 1. Parameter estimates (β_0 and β_1) of the models describing the effect of biofertilizer concentration on the mortality of *B. phoenicis* reared on *L. lucidum* plants and respective determination coefficients. Table 1.

Time (h)	Parameter	DF	Estimate	Standard error	x^2	*P* value *
24	β_0	1	-3.6331	0.5344	46.21	0.0001
	β_1	1	1.4562	0.3822	14.51	0.0001
	R^2	-	0.9752	-	-	-
48	β_0	1	-1.9737	0.3025	42.57	0.0001
	β_1	1	0.7157	0.2363	9.17	0.0025
	R^2	-	0.9868	-	-	-
72	β_0	1	-1.6483	0.2721	36.69	0.0001
	β_1	1	0.7096	0.2156	10.83	0.0010
	R^2	-	0.9929	-	-	-

Fig. 1 - Effect of biofertilizer concentration on the survival of *B. phoenicis* reared on *L. lucidum* leaves, 24, 48 and 72 h after spraying.

Figure 1.



The parameters of the adjusted models (β_0 e β_1) with their respective standard errors are presented in Table 1. The mite mortalities in the control ($e^{\beta 0}$) were 2.57, 12.20 e 16.13% for 24, 48 and 72 h, respectively, indicating that the mortality levels decreased with time. β_1 values decreased with time (Table 1) showing that the effect of the biofertilizer concentrations on the mite mortality was less intense in the course of time.

The estimated lethal concentrations (LC_{25}) were 50.04%, 15.70% and 4.95%, respectively, for the periods of 24, 48 and 72 hours after the pulverization (Table 2). Based on these values, it can be inferred that for killing 25% of the population in 72 hours it was necessary an amount of biofertilizer ten times smaller than the one used to cause the same mortality in 24 hours (LC_{25} 72:00 / LC_{25} 24 h).

Table 2. Lethal concentrations of the biofertilizer on the *B. phoenicis* reared on *L. lucidum* plants.
Table 2.

Time (h)	*No. of adults*	*LC_{25} (%)*
24	*352*	*50.04*
48	*352*	*15.70*
72	*352*	*4.95*

The biofertilizer presented low acute toxicity. However, in concentrations considered sub-lethal, chronic effects were observed in the population, suggesting smaller impact on non-target organisms. It is believed that with the slow and gradual reduction of the population of the phytophagous mite, there will be little interference in the trophic relationship between them and their natural enemies.

As there was not mortality superior to 50% in this bioassay, the fitted models fitting did not allow estimating LC_{50} values.

The daily number of eggs laid per female reduced with the increase of the concentration of the biofertilizer (β_1 = -0.0162; P = 0.0437) (Table 3 and 4), showing a negative interference of the biofertilizer on the reproduction rate of that species (Fig. 2).

Table 3. Mean daily number of eggs laid per *B. phoenicis* female reared on *L. lucidum* leaves, for each. Table 3.

Concentration	No. of mites	(Eggs/ female). day^{-1}
0	69	0.48
5	72	0.44
15	70	0.37
30	73	0.29
50	66	0.21

Table 4. * P-value associated to one-sided 't'-test.
Table 4. Estimates of the parameters β_0 e β_1 and determination coefficient (R^2) of the model that describes the effect of the biofertilizer on the oviposition of *B. phoenicis* reared on *L. lucidum* leaves with respective standard errors.
Table 4.

Parameters	DF	Estimate	Standard Error	t value	*P* - values*
β_0	1	-0.6724	0.1309	-5.136	0.0143
β_1	1	-0.0162	0.0048	-3.360	0.0437
R^2	-	0.8900	-	-	-

Contact action on mites reared on *Canavalia ensiformis.*

The mortality of *B. phoenicis* in response to the concentrations of the biofertilizer in *C. ensiformis* followed two different patterns. At 24 and 48 h after spraying, the mortality increased with the concentration (P=0.001) following exponential models (Fig. 3), with decreasing variation rates (Table 5). At the other evaluation times, (Fig. 4) the mortality also increased with the concentration (P<0.05), but the curves flattened off quickly (Table 6). For those cases, a fast increase in mortality was observed in the 0-5% concentration interval; there was no change in the concentration effect on mortality in the 10-30% concentration range (Fig. 4). A possible explanation for this fact is that no absorption occurred through the mite transcuticle. The high degree of ionization of the suspension, together with the large proportion of metabolites with high molecular weight, tended to increase its viscosity and the superficial tension (Kissmann 2002). Besides, the crystallization of the salts and other molecular compounds on the leaf contributed to that reduction. Those facts contributed to the decrease of the absorption of the active compounds of the biofertilizer by the mite, mainly those ones of larger molecular weight, when applied in high concentrations. Alves (1999) and Campos (2001), when using highly concentrated suspensions of chemical acaricides also observed similar effects.

Table 5. * P-values associated to likelihood ratio tests (x^2).
Table 5. Parameter estimates (β_0 and β_1) of the models describing the effect of biofertilizer concentration on the mortality of *B. phoenicis* reared on *C. ensiformis* plants for the times 24 and 48 h after Table 5.

Times (h)	Parameters	DF	Estimate	Standard Error	χ^2	*P* - values
24	β_0	1	-4.0917	0.5440	56.5644	0.0001
	β_1	1	3.1123	0.4486	48.1403	0.0001
	R^2	-	0.9867	-	-	-
48	β_0	1	-3.9955	0.5281	57.2442	0.0001
	β_1	1	3.0691	0.4376	49.1884	0.0001
	R^2	-	0.9852	-	-	-

Table 6. Parameters estimates (β_0, β_1 and $\beta 2$) of the models describing the effect of biofertilizer concentration on the survival of *B. phoenicis* reared on *C. ensiformis* plants for the times 72, 96 and 120 h after spraying and respective 95% asymptotic confidence intervals and determination coefficients (R^2) of the respective models.
Table 6.

Time (h)	Parameter	Estimate	Asymptotic Standard Error	95% Asymptotical confidence interval	
				Lower	Upper
72	β_0	0.0717	0.0713	-0.0766	0.2201
	β_1	0.5637	0.0933	0.3696	0.7579
	β_2	0.5985	0.8376	1.1434	2.3405
	R^2	0.9767	-	-	-
96	β_0	0.0873	0.0680	0.0540	0.2288
	β_1	0.5770	0.0887	0.3924	0.7616
	β_2	0.4818	0.5607	-0.6842	1.6479
	R^2	0.9805	-	-	-
120	β_0	0.1241	0.0690	-0.0194	0.2676
	β_1	0.5871	0.0909	0.3979	0.7762
	β_2	0.3827	0.3141	-0.2706	1.0359
	R^2	0.9863	-	-	-

There was an adequate fit of the model proposed for oviposition (R^2 = 97.97%); the parameter estimates of model of the neperian logarithm describing the mean number of eggs as function of the biofertilizer concentration is presented in Table 7.

Table 7. * Probability. Table 7. Parameters estimates (β_0 and β_1) of the models describing the effects of the biofertilizer on oviposition of the *B. phoenicis* reared on *C. ensiformis* plants and respective determination coefficients (R^2). Table 7.

Parameters	DF	estimate	Standard Error	t	*P* - values
β_0	1	-0.6008	0.0279	-21.489	0.0002
β_1	1	-0.0140	0.0016	-8.471	0.0035
R^2	-	0.9797	-	-	-

In a way similar to the results of the previous bioassay, there was significant effect (*P* <0.01) of the biofertilizer concentrations on the oviposition of *B. phoenicis* reared on *Canavalia* plants (Table 7). The daily number of eggs laid by females was reduced with the increase of the concentration, as indicated by the negative β_1 values (β_1 = -0,014028; *P* = 0,0035) (Tables 7 and 8). The exponential decrease of the oviposition, with the increase of the concentration, followed a pattern similar to the one observed in the bioassay on *L. lucidum* leaves. However, in this bioassay, a more pronounced effect of the concentrations was observed (Fig. 5).

Table 8. Mean daily number of eggs laid by *B. phoenicis* females reared on *C. ensiformis* plants, for each biofertilizer concentration. Table 8.

Concentration	*No. Females*	*eggs. female^{-1} daily*
0	*96*	*0.62*
5	*93*	*0.45*
10	*90*	*0.32*
20	*94*	*0.16*
30	*98*	*0.08*

The biofertilizer concentrations of 0.63 and 2.67% were enough to cause the 25 and 50% mortality, respectively, in *B. phoenicis* population in the 0-72 h period after spraying (Table 9).

The LC_{25} on *C. ensiformis* plants were similar to that ones obtained on *L. lucidum* leaves, in the previous bioassay. Then, considering that the 72 h-LC_{25} was 7.85 times less than the one obtained *L. lucidum* leaves, in laboratory conditions, it can be suggested some positive interference of the plant, increasing the power of lethal action of the biofertilizer compounds, a clear example of trophobiosis (Chaboussou 1985). Other important factor was that the values of LC_{25} and LC_{50} stabilized after 72 h (Table 9).

Table 9. Lethal concentrations LC_{25} and LC_{50} of the biofertilizer on *B. phoenicis* reared on *C. ensiformis* plants.
Table 9.

ne (h)	$_{25}$ (%)	$_{50}$ (%)
	15	.64
	78	.03
	53	38
	58	50
0	53	57

Several factors can explain the effects of the biofertilizer on the survival of *B. phoenicis*: intrinsic factors of the mite and plant, as well as mite vs plant interactions. *B. phoenicis* has presented control problems related to its high resistance degree to chemical acaricides. According to Helle (1980), this mite presents a reduced number of chromosomes (n = 2), favouring the selection of resistant genotypes, when submitted to selection pressure of one or more chemical products. In this way, *B. phoenicis* is a species with high probability of developing resistance to acaricides (Omoto 1995, Omoto et al. 2000, Campos 2001). Several bioecological factors can influence the resistance development in a population. According to Omoto (1998) the most important aspect is the reproduction mode. According to Helle (1980) and Weeks et al. (2001) *B. phoenicis* is the only haploid animal found in the nature that reproduces mainly, by thelytokous (obligate) parthenogenesis. In that reproduction mode, the offspring, originated from non-fertilized eggs are copies genetically identical to their progenitors. That phenomenon contributes significantly to the evolution of the resistance of a population (Omoto 1998). Considering the complexity of the biofertilizer composition (Santos 2001) and the mechanisms and factors related with the evolution of the resistance (Omoto 2000), it can be stated that there is a low probability of the mites developing multiple or cross-resistance to the active chemical components of the biofertilizer.

The biofertilizer efficacy on mite mortality can be synergically associated to a defense response of the plant that reacts simultaneously to the action of the biofertilizer (Deffune 2001). The mechanisms and/or compounds involved in that synergism are still not well known. Alterations that constitute the defense response of plants, caused by pathogens or herbivores have been studied aiming to improve the plant resistance (Kombrink and Somssich 1995, Dempsey et al. 1998, Gatehouse and Gatehouse 1998). Santos (2000) proposed that the odours of volatile substances, as alcohols, phenols and esters present in the biofertilizer, are responsible for the repellence and inhibition of feeding by insects and phythophagous mites. The feed habits of these herbivores would be strongly affected by abrupt changes in the composition of the plant tissues, induced by the biofertilizer.

Even with a short exposure time, it was observed a significant effect of the biofertilizer on mite fecundity pointing out that an abrupt physiologic alteration was trigged, altering the normal operation of the mite reproduction system. However, it is still unknown how the active biofertilizer compounds entered the mite body, if by ingestion and/or transcuticular absorption. Reduction in mite fecundity can also be related to the inhibition of the oviposition, by interference of chemical signs emitted by the plant and/or biofertilizer. As the mites were exposed to the topic and residual action simultaneously, it was not possible to be distinguished which action mode was the most efficient one. Omoto and McCoy (1998), testing a purified toxin of *Hirsutella thompsonii* on the mite *Phyllocoptruta oleivora* Ashm. (Acarina: Eriophyidae) verified a significant effect of concentration on fecundity, and that was more pronounced when the mite were exposed to the toxin only in a residual way. This information can have an important meaning for determination of the residual action of products, indicating how long the plant will be protected by the biofertilizer.

There is need to isolate and to quantify the effects of the defense response of the plant on the mite survival and reproduction. According to Mareggiani (2001) some important physiologic aspects, originating from of the coevolution mechanisms, especially those ones related to the presence of appropriate chemical receptors and the linked biochemical aspects, that can repress the capacity of the herbivores to excrete, biodegrade and to sequester toxic metabolites, destined to avoid the attack of predators and defense of the herbivores against the pathogens action. Schoonhoven et al. (1998) showed that the chemical activity of the secondary metabolites of the plant is varied and that many of them possess biological activity on insects, altering their feeding, development and reproduction or behaviour. The molecular composition of the biofertilizer is of primary and secondary metabolic origin coming from different classes of microorganisms, with functions that vary on the plant. They can work as feed deterrents, growth regulators and inhibitors of insect and mite oviposition (Santos and Akiba 1996).

Pathological action of the biofertilizer on *B. phoenicis*.

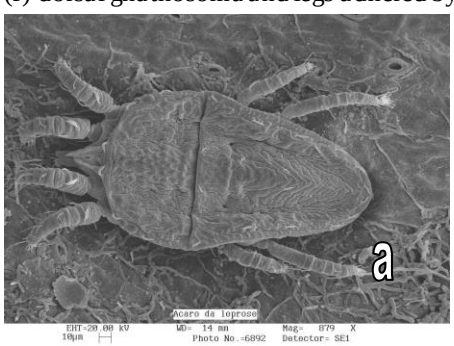
In the treated plants, it was verified that some mites walked, in a guideless way, inside of the contention glue, an evidence that, probably, they had its nervous system altered, disabling them to avoid of the glue. This behaviour was not observed in the control plants. Guirado (1999), evaluating different plant extracts as control agents on the same mite, observed a similar behaviour. It was suggested that the compounds of the extracts acted as repellents to the *B. phoenicis*.

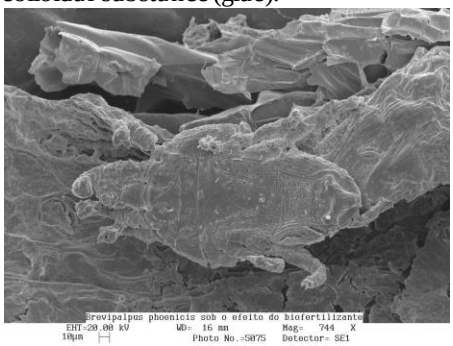
The cadavers coming from the control did not present morphological alterations, 24 hours after the death (Fig. 6). In the treated plants, the mites dead by biofertilizer were laid in the horizontal position, with the back turned down. This behaviour is considered uncommon: it was never observed when the mites were killed by chemical acaricides action. It can be explained by the constants attempt of the mite in releasing itself from the colloidal compound of the

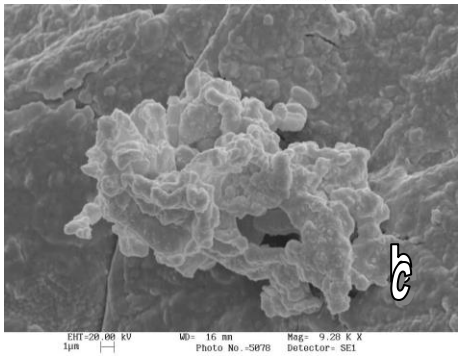
biofertilizer. During the experiment it was verified that, hours after having sprayed, the mites became agitated and possibly stopped feeding.

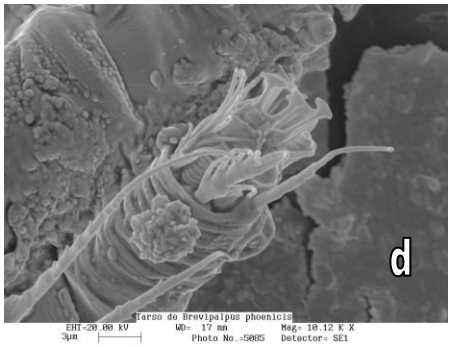
Evidences of microbial colonization, like presence of external mycelia on the cadavers of *B. phoenicis* killed by action of the biofertilizer, were verified. In addition to the unusual position of the cadaver, some structures of the mite such as oral apparatus and legs were damaged. The legs were adhered to a colloidal substance and there were microbial structures on the metapodosoma, microbial focus in the tarsus and in the genital plate (Fig. 6).

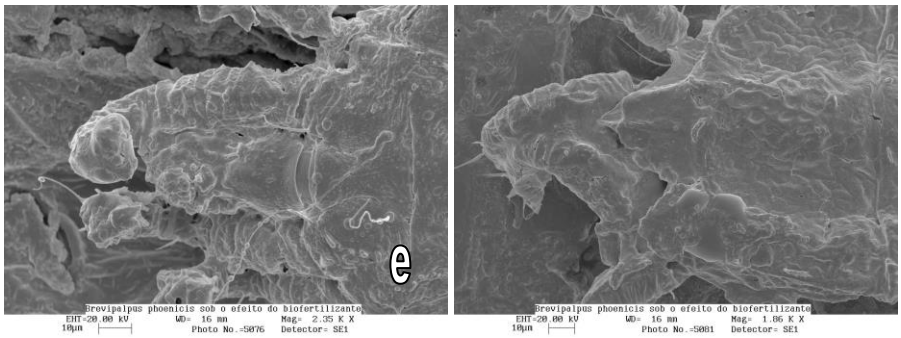
Fig. 6 – Caracterization of *B. phoenicis cadavers*: (a) control, (b) Mite dead by contact biofertilizer action. (c) Microbial growing in the metapodosomal medium ventral integument, (d) Microbial focus in the tarsus and (e) ventral and (f) dorsal gnathosoma and legs adhered by a colloidal substance (glue).











The colloidal substance had a mucilaginous appearance, probably a gum of secondary metabolic origin. Due to its viscous consistence and high molecular weight (polymeric), this gum is not absorbed by the cellular wall of the plant or by the mite cuticle. Its composition is still unknown; it acted mechanically, causing the legs and the gnathosoma to adhere and the digestive tract to obstruct. Others functional opening were also filled by the gum. The mites were immobilized on the foliar surface until they died. Colloidal substances, showing similar mechanisms of action, were also reported by Guirado (1999), who observed that the adults of *B. phoenicis* sprayed with alicina did not feed and died agglutinated on the leaves. Santos (2001) described the same effect of biofertilizers on aphids and other hemipteroids when sprayed with biofertilizers in concentrations superior to 50%.

Based on the experimental results, the conclusions are: (i) the bioassay method accomplished directly on the plant is adequate for contact effect evaluation of the biofertilizer on *B. phoenicis*; (ii) the deleterious effect of the biofertilizer on the reproduction and survival of *B. phoenicis* was proportional to he concentration; (iii) the biofertilizer contains a mucilaginous substance that causes the adult *B. phoenicis* to die and (iv) the biofertilizer is a promising tool for ecological management of *B. phoenicis* populations in the Brazilian organic agriculture.

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