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Effect of Disinfectants and Pesticides Used in Poultry Houses on Beauveria Bassiana (Bals.) Vuill.fungus

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■Keywords

Entomopathogenic fungus. Compatibility. Associated control. Animal production.

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ABSTRACT

This study aimed at evaluating the effect of the use of disinfectants and insecticides recommended for the control of pathogens and insects in poultry houses on the biological parameters of the entomopathogenic fungus *Beauveria bassiana* strain Unioeste 4. Disinfectants and insecticides were used at recommended concentration (RC), half the recommended concentration (0.5 RC), and twice there commended concentration (2RC). All treatments were sprayed on the fungus previously inoculated on PDA culture media. Germination, colony forming unit (CFU), vegetative growth and conidial yield were evaluated. Germination was the most affected parameter by insecticides, with reduction of up to 25% using Couro Limpo and Cypermil products. Cypermil also reduced the vegetative growth in all tested concentrations. Conidial production was reduced when products were used at the highest concentration. On the other hand, the confirmed mortality of the lesser mealworm by the fungus was not affected by none of the tested products. All disinfectants at the highest concentration reduced germination and conidia production, but did not affect fungus vegetative growth. The confirmed mortality by the fungus was most influenced by the product TH4. All tested products tested were considered compatible with the fungus.

INTRODUCTION

The intensive production of broilers is characterized by the confinement of large numbers of individuals in a small area, which provides high productivity. However, these houses, together with the presence of organic waste (from feed and feces), provide favorable shelter, temperature, light, and moisture conditions for the development of large populations of the lesser mealworm (*Alphitobius diaperinus* Panzer) (Coleoptera: Tenebrionidae) and sources of the inoculum of *Salmonella*. These can reduce flock productivity and damage the health of animals and of the humans involved in the production chain, including consumers. It is emphasized that these species have a prominent place in poultry industry worldwide, and cause significant losses, and therefore, need to be eradicated (Axtell, 1999; Silva & Duarte, 2002; Brasil, 2003; Penha, 2008; Hazeleger *et al*., 2008; Omalu *et al*., 2012).

Despite the current poultry management practices recommended for the control and elimination of pathogens, little progress has been made in the search of alternatives to the use of chemical disinfectants, which are commonly applied, such as quaternary ammonium compounds, glutaraldehyde, sodium hypochlorite, and organic acids (Jaenisch *et al*., 2010).

Chemical control is the main strategy applied for the control of the lesser mealworm. The most common products are pyrethroid insecticides, which are continuously applied, with or without poultry litter (Santos *et al*., 2009). However, many natural enemies of the lesser

mealworm are found in poultry houses, including predators and entomopathogenic fungi (Alves *et al*., 2004; 2005; Santoro *et al*., 2010). The potential of such fungi for lesser mealworm control was demonstrated both under laboratory and field conditions (Geden *et al*., 1998; Rohde *et al*., 2006, Chernaki *et al*., 2007). In addition, it is safe for poultry (Hass *et al*., 2010).

However, poultry house management practices may interfere with the activity and survival of the fungus. Previous studies showed that litter chemical composition, structure, and temperature may negatively affect fungus survival, and therefore, its activity against both lesser mealworm larvae and adults (Bacon, 1985; Alexandre *et al*., 2006; Alves *et al*., 2008). In our previous studies with *Beauveria bassiana* isolate Unioeste 4 applied to the soil or combined with poultry litter, the population of lesser mealworm was reduced in 80% (Alves *et al*., 2015).

When used for the control of pests, fungi are also part of the poultry management system, as well as chemical disinfectants and insecticides (Bellaver *et al*., 2003). However, such chemicals must be rationally applied, i.e., the preservation of natural pest enemies and the health of poultry and humans must be taken into account. Although many studies on the impact organic-synthetic pesticides on natural pest enemies were published, no such articles were found in the context of poultry production, nor specifically on the fungus *B. bassiana*. Therefore, the objective of, this experiment was to evaluated the effects of disinfectants and insecticides commonly used in poultry houses on the fungus *B. bassiana* strain Unioeste 4 in order make recommendations for their application.

MATERIAL AND METHODS

Chemical products

Commercial disinfectants and insecticides registered for poultry house application were used at the concentration recommended by the manufacturer (RC), half the recommended concentration (0.5 RC), or twice the recommended concentration (2RC) (Table 1).

Microorganism

Beauveria bassiana isolate Unioeste 4 was obtained from culture collection of Laboratório de Biotecnologia Agrícola da Unioeste (http://splink.cria.org.br/manager/detail?resource=CFEUnioeste). Fungal conidia were obtained from 10-day-old colonies grown in Petri dishes with culture medium (20 g agar, 5 g yeast extract, 4.6 g salt mixture, 10 g glucose, and 1000 mL distilled water).

Table 1 – Products used in the experiments and their respective composition, as described from manufacturer.

Brand name	Composition[1]
Insecticides	
Couro Limpo	Cypermethrin 15g, Chlorpyrifos 25g; Citronellal 1 g, q.s.p. vehicle 100 mL - 25mL/20L
Colosso	Cypermethrin 15g, Chlorpyrifos 25g; Citronellal 1 g, q.s.p. vehicle 100 mL - 25mL/20L
Cypermil	Cypermethrin 15 g; vehicle q.s.p. 100 mL - 20 mL/20L
Disinfectants	
Aviclor	1,3,5 triazine, 2,4,6 trione, 1,3 dichlor sodium 75%; adjuvant 0.248%; excipient 22.52% - 1 kg / 1000L–1kg/1000 L
AVT 80	Benzalkonium chloride 64%; q.s.p. vehicle 36% - 250mL/1000L
CB–30 TA	Benzalkonium chloride 30g, nonyl phenoxy polyethoxy ethanol 5g; vehicle q.s.p. - 10mL/20L
Glutasil	Glutaraldehyde 42,5%, benzalkonium chloride 7,5%, vehicle q.s.p. 100 mL - L 1L/100
Glutaquat	Glutaraldehyde 40g, benzalkonium chloride 10 g, vehicle q.s.p. 100 mL - L 1L/1000
Kilol®	1 mL ascorbic acid, 0.475 mL citric acid, 0.47 mL lactic acid, water q.s.p. 100mL - 1L/250L
TH4	Didecyldimethylammonium chloride 1.87g; dioctyldimethylammoniumchloride 1.87 g; octyldecyldimethylammonium chloride 3.7 g; alkyldimethylbenzylammonium chloride 5g, glutaraldehyde 6.2 g; pine oil 2g, terpineol 2g; vehicle q.s.p.100mL - 1L/200L

[1] Information provided by the manufacturers

B. bassiana biological parameters

The following biological parameters were used to evaluate the effects of the applied chemical products on *B. bassiana*: conidial germination, colony forming units (CFU), vegetative growth, and conidial production (Alves *et al*., 1998a; Silva *et al*., 2005; Oliveira, 2009).

A) Germination: 300 µL of a conidial suspension (1×10^6 conidia/mL) was inoculated onto PDA culture media in Petri dishes, which were lightly shaken to spread the suspension. Then, 250 µL of the solutions of the chemical products were sprayed onto the surface of the culture media with an airbrush coupled to a continuous airflow compressor (0.84 kgf/cm^2). Dishes were incubated at 26 °C for 16 h under a12-h photoperiod, after which germinated and non-germinated conidia were counted under an optical microscope totaling, on average, approximately 200 conidia/plate; the presence of germ tubes (showing at least twice the conidial diameter) was considered.

B) Colony Forming Units (CFU): 100 µL of a conidial suspension (1×10^3 conidia/mL) were inoculated according to the same procedures as described above, and incubated for five days, after which the colonies formed were counted.

C) Vegetative growth: the fungus was inoculated using a platinum loop at 3 points on the surface of

potato dextrose agar (PDA) culture medium, incubated at 26 °C under a 12-h photoperiod for 48 h, after which the chemical products were sprayed. Plates were again incubated under the same conditions for 7 days. Two perpendicular measures of the colonies were performed to evaluate vegetative growth.

D) Conidia production: after evaluating vegetative growth, a colony in each plate was cut and transferred to a sterile glass flasks containing sterilized distilled water + 0.01% Tween® 80. Flasks were agitated for 1 min and conidia were counted in a Neubauer chamber.

A completely randomized experimental design with insecticides and disinfectants with four replicates each was applied. Data were submitted to analysis of variance (F test) and means were compared by the Scott Knott test at 5% significance level, using Sisvar software (Ferreira, 2011).

The effect of the evaluated products on the fungus was evaluated according to the equation proposed by Rossi-Zalaf *et al*., (2008), as: BI: 47[CV] + 43[ESP] + 10[GER]/100, where: IB = Biological Index, CV = vegetative growth percentage of the colony after 7 days relative to the control, ESP = percentage of sporulation of colonies after 7 days compared with the control; GER = percentage of conidia germination after 16 h. The BI values (p=0.05) used for products classification were: Toxic 0-41, Moderately Toxic 42-66, and Compatible>66.

E) Insecticidal activity of *B. bassiana:* Conidia were applied to the surface of the PDA culture medium, spread with a Drigalski handle, and then sprayed with 250 µL of each product/plate. Plates were incubated for 7 days at 26 ± 1 °C and 12-h photoperiod. Conidia were collected and the suspensions were prepared (1 × 10^8conidia/mL) in a glass tube according to Rohde *et al*., (2006). Adults of the lesser meal worm were immersed in the suspensions and, after manual stirring for 1 minute, were transferred to Petri dishes to remove fluid excess and transferred to other Petri dishes containing poultry feed.

One plate was not sprayed and used as control. Insects were immersed as described above in distilled water + 0.01 Tween® 80. The insects were kept at 26 ± 1 °C and a 12 h photophase. Daily mortality was assessed for 10 days. Dead insects were immersed in 70% ethanol and then in distilled water and kept in a moist chamber (confirmed mortality by the fungus), observing signs and symptoms of fungal infection as described by Alves *et al*., (1998b).

For each product, five plates with 15 adults were prepared, and each plate was considered a replicate. Data were analyzed for normality by the Shapiro-Wilk test and transformed when necessary. Subsequently, data were submitted to analysis of variance (F test) and means were compared by the Scott-Knott test (5% significance level) using Sisvar software.

RESULTS AND DISCUSSION

Insecticides

All products tested significantly reduced conidial germination, notably Couro Limpo and Colosso products, both at the recommended concentration (RC), but there was no effect on CFU. Colony diameter was significantly reduced by Couro Limpo and Colosso products, both at 2RC, and by Cypermil at all concentrations tested. Conidial production was significantly lower only when products were used at2 RC. However, all products evaluated were considered compatible with fungus, with BI values higher than the minimum considered (Table 2).

Table 2 – Mean values of biological parameters and biological index of the fungus *Beauveria bassiana* (strain Unioeste 4) obtained in culture medium containing insecticides (26 ± 2 °C, RH: 60 ± 10%; photophase: 14h).

Products	Germination (%)	CFU	Diameter (cm)	conidia (× 10^6 / mL)	BI[2]	Total mortality (%)	Confirmed mortality (%)
Fungus	100.00 a	202.75 a	2.67 a	58.95 a	-	100.0 ± 0.0 a	88.0 ± 3.8 a
Couro limpo (0.5RC)	88.32 b	213.75 a	2.68 a	45.85 a	80.37 C	90.6 ± 3.4 b	84.0 ± 4.99 a
Couro limpo (RC)	75.01 c	202.50 a	2.71 a	48.63 a	82.28 C	98.6 ± 1.3 a	90.6 ± 4.9 a
Couro limpo (2RC)	83.10 b	213.25 a	2.59 b	44.09 b	76.61 C	100.0 ± 0.0 a	96.0 ± 2.6 a
Colosso (0.5RC)	85.60 b	202.00 a	2.68 a	54.07 a	91.90 C	98.6 ± 1.3 a	93.3 ± 5.1 a
Colosso (RC)	75.91 c	229.50 a	2.69 a	52.93 a	81.49 C	94.6 ± 2.4 b	81.3 ± 4.4 a
Colosso (2RC)	80.76 b	218.50 a	2.61 b	39.04 b	79.31 C	94.6 ± 1.3 b	87.9 ± 3.27 a
Cypermil (0.5RC)	84.56 b	226.00 a	2.62 b	54.60 a	89.97 C	91.9 ± 3.2 b	85.3 ± 6.4 a
Cypermil (RC)	85.24 b	192.50 a	2.52 c	51.99 a	83.90 C	100.0 ± 0.0 a	93.3 ± 5.16 a
Cypermil (2RC)	82.56 b	204.00 a	2.53 c	34.04 b	71.09 C	98.6 ± 1.33 a	93.3 ± 2.9 a
C.V.	5.59	15.32	1.75	15.26	-	4.91	11.92

Information collected from manufacturers

Means (± MSE) followed by the same letter in the column do not differ by the Scott Knott test (p<0.05)

[1]Products concentrations: 0.5 RC = Half the recommended concentration, RC = Recommended Concentration; 2RC = Twice the recommended concentration.

[2]IB = biological index, according to Rossi-Zalaf *et al*., (2008): 0 to 41 = toxic (T); 42-66 = moderately toxic (MT); greater than 66 = compatible (C)

Germination it was the biological parameter that was most affected by insecticides. Conidial germination is essential to initiate the infectious process in the host, and it is more important than vegetative growth or conidial production of *B. bassiana* (Feng *et al*.,1994; Todorova *et al*., 1998).

The objective of the applied methodology was to simulate actual conditions of fungus exposure to the chemical products, as indicated by Oliveira & Neves (2004), and therefore, the responses obtained in the present experiment actually express the interaction between the fungus and the products and their impact on the evaluated parameters.

The negative effect of cypermethrin on colony diameter was also verified by Lecuona *et al*., (2001), when evaluating associations of *B. bassiana*withchemical insecticides for the control of *Triatoma infestans*. Those authors reported that such associations are only possible when insecticides were applied at 10% of the recommended concentration.

It is difficult to explain the different results obtained with insecticides, since cypermethrin is the active ingredient of three tested products. On the other hand, the similar effects of Couro Limpo and Colosso on all parameters may be explained by their identical composition.

Maintaining the integrity of the outer surface of the conidia is important for the germination process. The components of the chemical products may affect such integrity, and therefore, fungal germination (Boucias *et al*., 1988;St. Leger *et al*., 1991). Germination may also be reduced when certain molecules bind to receptors in the fungal cytoplasm, affecting membrane permeability and enzyme synthesis, changing conidial metabolism (Moore-Landecker1982; Ghini & Kimati 2000; Oliveira & Neves, 2004).

The evaluated insecticides had no effect on colony formation (CFU), indicating that the metabolism was not affected at this level. Although it is likely that the products applied on the medium surface, being in contact with the cells of the fungus, have been widely spread, were absorbed by the fungus and accumulated in the cytoplasm over five days of incubation; however, concentration required to inhibit fungal metabolism was probably not reached (Kimati, 1995).

The incubation time used to evaluate vegetative growth was higher than CFU incubation time, demonstrating a direct relation of the insecticides on vegetative growth as contact time increased. It is possible that the chemical compounds accumulated in the fungal cytoplasm, and affected its enzymatic apparatus. It is noteworthy that the 2 RC concentrations is not applied in field conditions, and *in-vitro* assay shows an extreme contact. Also, mycelial growth stage develops inside the body of the insect host, where the insecticidal concentrations are usually low, negative effects of insecticides on this developmental stage are unlikely (Khalil *et al*., 1985).

The insecticidal activity of the fungus produced when insecticides were applied on the culture media was reduced (total mortality was 10% lower than control) with Couro Limpo and Cypermil, both at 0.5 RC, and with Colosso at 1 and 2 RC. However, there were no differences in confirmed mortality when comparing the fungi grown in culture media either with or without the evaluated products (Table 2).

Therefore, it is possible the effects of either the active ingredients or adjuvants in the insecticide formulations on fungal germination or growth did not cause any changes in the synthesis toxins or enzymes, which are important for host colonization by the fungus (Xiao *et al*., 2012), as shown by the lack of differences in confirmed mortality. On the other hand, the absence of effect on confirmed mortality may also indicate that the fungus developed on a substrate (insect cadaver) where the evaluated products were not present, therefore precluding any negative on the fungus.

Although the total mortality of insects inoculated with fungi grown in insecticide-containing media was reduced, this result should not be understood as reduced fungal efficiency caused by the insecticides, as the objective was merely to assess the *in-vitro* biological effects of the insecticides on fungal activity. Under field conditions, the concentrations of insecticide in the insect body are much lower than those used in the culture media, and therefore, the application of the insecticides tested in the present experiment may not impact the fungus present in poultry houses (in case of natural occurrence or to control the lesser mealworm). On the other hand, the fungi may also remain in the environment, despite the effects observed here, as they multiply in insect cadavers that may not have been affected by the chemical products.

Agricultural pyrethroid insecticides are evaluated as a function of their positive and negative inhibitory actions on entomopathogenic fungi, and may vary according to product concentration, active ingredient, formulation, and type of adjuvant, as well as to the evaluation technique and fungal strains and species involved (Lecuona & Diaz, 2001; Lecuona *et al*., 2001; Barci *et al*., 2009). However, as there are no reports of such studies with poultry, conclusions of such studies are only valid under the conditions of each experiment and should not be generalized.

Disinfectants

All evaluated disinfectants reduced fungal germination, specially at RC and 2 RC, in relation to the controls. In particular, the products AVT80 and Glutasil at RC and 2RC reduced conidial germination between 20 and 25%.

There were no significant effects on vegetative growth parameters (CFU formation and colony diameter), as previously observed with the insecticides (Table 3).

On the other hand, conidial production/colony was greatly affected by AVT80 at 2RC, CB-30 at 2RC, Glutaquat at RC and 2RC (50%), and Kilol at RC and 2 RC (40%).

Despite these values, all disinfectants, as observed with insecticides, were compatible with the fungus, with BI values above the minimum required for them to be considered compatible.

Overall, the effects of disinfectants on fungal viability and vegetative growth were less severe than those observed with insecticides, although both have antimicrobial activity.

The product Glutaquat (at all concentrations) and Glutasil (at 2RC) showed an inhibitory effect on conidial production/colony. Glutaraldehyde acts on fungi, especially on the cell walls, interacting with chitin, promoting injury (McDonnell & Russell, 1999;Ristow, 2008). Benzalkonium chloride present in the AVT80 and CB30 products (main compound) and Glutaquat and Glutasil at 2RC (minor compound) is a cationic surfactant that modifies the lipid structure of the surface plasma membrane of the conidia, thereby affecting germination (McDonnell & Russell, 1999; Ristow, 2008).

The observed absence or weak effects of the tested disinfectants on *B. bassiana* germination, CFU, and diameter may be ascribed to the metabolism of this fungus, which has a rich enzymatic apparatus and may have broken down the molecules of the tested products (Moino Jr. & Alves, 1998; Silva & Espósito, 2004). In addition, the diffusion capacity of the compounds tested in the culture media may have been low, therefore the contact with the fungus may have been reduced. As Anderson & Roberts (1983) demonstrated that the

Table 3 – Biological parameters and biological index of the fungus *Beauveria bassiana* (strain Unioeste 4) obtained in culture medium containing disinfectant products (26 ± 2 °C, RH: 60 ± 10%; photophase: 14h).

Products	Germination (%)	CFU	Diameter(cm)	conidia (× 10^6 / mL)	BI[2]	Total mortality (%)	Confirmed mortality (%)
Disinfectants							
Control	98.3 a	44.75 a	2.65 a	69.0 a	-	88.0 ± 3.8 b	88.0 ± 3.8 a
Aviclor (0.5RC)	91.8 a	34.50 a	2.61 a	61.9 a	87.1 C	100.0 ± 0.0 a	100.0 ± 0.0 a
Aviclor (RC)	75.5 b	43.25 a	2.63 a	52.6 b	79.0 C	95.9 ± 1.6 a	81.3 ± 7.4 b
Aviclor (2RC)	86.5 a	40.25 a	2.56 a	46.9c	77.0 C	86.6 ± 3.6 b	86.6 ± 3.6 a
AVT 80(0.5RC)	91.8 a	35.25 a	2.63 a	43.8 c	81.2 C	90.6 ± 1.6 b	90.6 ± 1.6 a
AVT 80(RC)	75.5 b	43.25 a	2.63 a	39.9 c	72.2 C	93.3 ± 4.2 b	92.0 ± 5.3 a
AVT 80 (2RC)	80.7 b	41.00 a	2.63 a	36.4 d	71.2 C	100.0 ± 0.0 a	100.0 ± 0.0 a
CB–30 TA (0.5RC)	93.1 a	41.50 a	2.59 a	55.7 b	82.5 C	98.6 ± 1.3 a	96.0 ± 2.6 a
CB–30 TA (RC)	86.6 a	47.00 a	2.58 a	43.2 c	67.0 C	100.0 ± 0.0 a	100.0 ± 0.0 a
CB–30 TA (2RC)	79.6 b	42.00 a	2.58 a	33.0 d	68.0 C	98.6 ± 1.3 a	98.6 ± 1.3 a
Glutaquat (0.5RC)	89.9 a	42.00 a	2.66 a	44.6 c	78.5 C	95.9 ± 1.6 a	68.0 ± 8.2 b
Glutaquat (RC)	92.8 a	43.25 a	2.59 a	36.8 d	63.4 C	100.0 ± 0.0 a	100.0 ± 0.0 a
Glutaquat (2RC)	76.3 b	40.00 a	2.56 a	37.1 d	65.5 C	93.3 ± 3.6 b	79.9 ± 10.3 b
Glutasil 50 (0.5RC)	88.8 a	45.75 a	2.57 a	58.5 a	87.3 C	98.6 ± 1.3 a	98.6 ± 1.3 a
Glutasil 50 (RC)	72.9 b	39.00 a	2.67 a	58.4 a	86.7 C	100.0 ± 0.0 a	86.6 ± 11.4 a
Glutasil 50 (2RC)	79.9 b	41.50 a	2.54 a	42.8 c	68.9 C	89.3 ± 4.9 b	89.3 ± 4.9 a
Kilol® ((0.5RC)	99.1 a	47.25 a	2.62 a	39.9 c	73.4 C	100.0 ± 0.0 a	93.3 ± 5.1 a
Kilol® (RC)	93.5 a	37.25 a	2.61 a	30.1 d	66.4 C	100.0 ± 0.0 a	77.3 ± 10.8 b
Kilol® (2RC)	84.3 b	42.50 a	2.73 a	27.0 d	66.5 C	96.0 ± 4.0 a	96.0 ± 4.0 a
TH4 (0.5RC)	84.3 b	49.00 a	2.57 a	49.17 b	81.8 C	100.0 ± 0.0 a	80.0 ± 12.2 b
TH4 (RC)	89.0 a	42.25 a	2.49 a	49.35 b	80.4 C	100.0 ± 0.0 a	77.3 ± 14.0 b
TH4 (2RC)	80.8 b	47.50 a	2.62 a	43.80 c	70.7 C	90.6 ± 3.4 b	90.6 ± 3.4 a
C.V.	7.78	22.88	2.84	17.74	-		

Means (± SEM) followed by the same letter in the same column are not different by the Scott Knott test ($p<0.05$)
[1]Products concentrations: 0.5 RC = half the recommended concentration, RC = recommended Concentration; 2RC = twice the recommended concentration.
[2]IB = biological index, according to Rossi-Zalaf *et al.*, (2008): 0 to 41 = toxic (T); 42-66 = moderately toxic (MT); greater than 66 = compatible (C)
RC = recommended concentration; C.V.= Coefficient of Variation; CFU=Colony Forming Units

ingredients included in the disinfectant formulation may be more important than the active ingredients, allowing conidial dispersion on the surface of the medium during inoculation, promoting the development of a higher number of CFU.

In contrast, conidial production was reduced with most of the products tested, except for Aviclor 0.5 RC and Glutasil 0.5 RC and 2 RC. Kilol reduced conidial production by almost 50%. Despite not having influenced other fungal parameters, the disinfectants may have affected some important metabolic steps in conidial production, as shown by McDonnell & Russel (1999) in an extensive review on the mode of action of synthetic disinfectants.

Although fungal growth was not affected, it is possible that the production of toxins and enzymes important for the colonization process was negatively affected, as shown by the reduction in total and confirmed mortality (Table 4), as previously discussed in the insecticides.

Table 4 – Mortality percentage (± SEM) of adults of *Alphitobius diaperinus* by *Beauveria bassiana* fungus (strain Unioeste 4) obtained in culture medium containing disinfectants (26 ± 2 ºC, RH: 60 ± 10%; photophase: 14h).

Treatment	Total mortality (%)	Confirmed mortality (%)
Control	6.6 ± 5.1 c	0.0 ± 0.0 c
Fungus	88.0 ± 3.8 b	88.0 ± 3.8 a
CB-30 0.5RC	100.0 ± 0.0 a	100.0 ± 0.0 a
CB-30 RC	95.9 ± 1.6 a	81.3 ± 7.4 b
CB-30 2RC	86.6 ± 3.6 b	86.6 ± 3.6 a
Glutasil 0.5RC	90.6 ± 1.6 b	90.6 ± 1.6 a
Glutasil RC	93.3 ± 4.2 b	92.0 ± 5.3 a
Glutasil 2RC	100.0 ± 0.0 a	100.0 ± 0.0 a
TH4 0.5RC	98.6 ± 1.3 a	96.0 ± 2.6 a
TH4 RC	100.0 ± 0.0 a	100.0 ± 0.0 a
TH4 2RC	98.6 ± 1.3 a	98.6 ± 1.3 a
Aviclor 0.5RC	95.9 ± 1.6 a	68.0 ± 8.2 b
Aviclor RC	100.0 ± 0.0 a	100.0 ± 0.0 a
Aviclor 2RC	93.3 ± 3.6 b	79.9 ± 10.3 b
Glutaquat 0.5RC	98.6 ± 1.3 a	98.6 ± 1.3 a
Glutaquat RC	100.0 ± 0.0 a	86.6 ± 11.4 a
Glutaquat 2RC	89.3 ± 4.9 b	89.3 ± 4.9 a
Kilol 0.5RC	100.0 ± 0.0 a	93.3 ± 5.1 a
Kilol RC	100.0 ± 0.0 a	77.3 ± 10.8 b
Kilol 2RC	96.0 ± 4.0 a	96.0 ± 4.0 a
AVT-80 0.5RC	100.0 ± 0.0 a	80.0 ± 12.2 b
AVT-80 RC	100.0 ± 0.0 a	77.3 ± 14.0 b
AVT-80 2RC	90.6 ± 3.4 b	90.6 ± 3.4 a
C.V. (%) =	6.23	17.08

Means (± SEM) followed by the same letter in the column do not differ by the Scott Knott test ($p<0.05$)
RC = recommended concentration; C.V. = Coefficient of Variation

The multiplication of either naturally-occurring or applied fungi in dead insects allows their permanence in poultry houses and should be preserved (Alves, 1998). Therefore, when using disinfectants in poultry houses, care must be taken to preserve those populations (Steinkraus *et al*., 1991; Alves *et al*., 2004; 2005).

In addition, it should be noted that the observed effects on fungal activity does not correspond to the reduced efficiency of a combined application of insecticides or disinfectants and the fungus. Alexandre *et al*. (2008), using the Colosso insecticide and *B. bassiana* Unioeste 4 strain, showed that mixtures of fungus and insecticides could be used only when the fungus was used at concentrations equivalent to CL_{70}; the insecticide at the concentrations of CL_5 and CL_{10} showed an additive effect. When the insecticide concentration was higher than that of the fungus, fungal activity was reduced.

So, it may be inferred that the fungal application in poultry house may be affected by the presence of the products here evaluated. However, the reduction in the inoculum potential (as that observed with the chemical treatment of the poultry house) may be overridden by fungus reapplications. In addition, the ability of the *B. bassiana* fungus to grow saprophytically in the environment and to produce secondary inoculum sources through sporulation on cadavers may potentially be used for the conservation of that entomopathogen in poultry houses.

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