

PEREIRA, AIA; SILVA, CM; CURVÊLO, CRS; PONTES, NC; PEREIRA, JL; TAVARES, WS; ZANUNCIO, JC; LUZ, JMQ. 2020. Mixtures between *Beauveria bassiana* and potassium silicate to manage thrips in tomato plants for industrial processing. *Horticultura Brasileira* 38: 415-420. Doi: <http://dx.doi.org/10.1590/s0102-0536202004012>

Mixtures between *Beauveria bassiana* and potassium silicate to manage thrips in tomato plants for industrial processing

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ABSTRACT

Thysanoptera species can transmit virus to Solanaceae family vegetables, including tomato plants for industrial processing, causing the disease known as Tomato Spotted Wilt Virus (TSWV). Thrips resistance to insecticides indicate the urgent need of techniques adequate for Integrate Pest Management practices. The objective was to evaluate the control efficiency (CE%) against *Frankliniella schultzei* (Thysanoptera: Thripidae) of the fungus *Beauveria bassiana* (Bb), the resistance inducer potassium silicate (KSil) and the chemical insecticide profenophos + cypermethrin (PC) isolated, or in binary mixtures. The treatments were foliar spraying on tomato plants with Bb (T1), KSil (T2) and PC (T3) isolated or in mixtures of Bb+KSil (T4), PC+KSil (T5) and Bb+PC (T6). Thrips were sampled with white plastic trays at 0, 1, 7, 14 and 21 days after application (daa). The CE% of each product (isolated or in mixtures) differed at all time intervals. The Bb+KSil treatment had highest CE% from 24 hours of application, until the end of the experiment, ranging from 95% (1 daa) to 41.5% (21 daa). The CE% decreased in all treatments with a quadratic curve behavior, throughout time. PC insecticide, isolated, showed a decreased CE% through a linear regression. Grouping analyzes indicated the Bb+KSil effect was more dissimilar than the other evaluated treatments. Synergism between Bb and KSil, in mixture, indicate its potential for Integrated Pest Management programs of *F. schultzei* in tomato plants for industrial processing.

Keywords: *Frankliniella schultzei*, *Solanum lycopersicum*, entomopathogenic fungi, silicon, synergism, tank mixture.

RESUMO

Misturas entre *Beauveria bassiana* e silicato de potássio para o manejo de tripses em plantas de tomate para processamento industrial

Espécies de Thysanoptera podem transmitir vírus para vegetais da família Solanaceae, incluindo plantas de tomate para processamento industrial, causando a doença vira-cabeça do tomateiro (TSWV). A resistência dos tripses a inseticidas indica a necessidade urgente de técnicas adequadas às práticas de Manejo Integrado de Pragas. O objetivo foi avaliar a eficiência de controle (CE%) de *Frankliniella schultzei* (Thysanoptera: Thripidae) com o fungo *Beauveria bassiana* (Bb), o indutor de resistência silicato de potássio (KSil) e o inseticida químico profenofós + cipermetrina (PC) isolados, ou em misturas binárias. Os tratamentos foram aplicados via foliar nas plantas de tomate com Bb (T1), KSil (T2) e PC (T3) isolados ou em misturas binárias Bb+KSil (T4), PC+KSil (T5) e Bb+PC (T6). Os tripses foram amostrados com bandejas plásticas aos 0, 1, 7, 14 e 21 dias após a aplicação (daa). A CE% de cada produto (isolado ou em misturas) diferiu em todos os intervalos de tempo. O tratamento Bb+KSil teve alta CE% desde as primeiras 24 horas da aplicação até o final do período experimental, variando entre 95% (1 daa) a 41,5% (21 daa). A CE% diminuiu em todos os tratamentos com um comportamento quadrático ao longo do tempo. O inseticida PC, isolado, apresentou uma diminuição para a CE% através de uma regressão linear. Análises de agrupamento indicaram que o efeito do Bb+KSil foi mais heterogêneo do que o dos outros tratamentos avaliados. O sinergismo entre Bb e KSil, em mistura, indica seu potencial em programas de Manejo Integrado de Pragas de *F. schultzei* em plantas de tomate para processamento industrial.

Palavras-chave: *Frankliniella schultzei*, *Solanum lycopersicum*, fungos entomopatogênicos, silício, sinergismo, mistura em tanque.

Received on May 5, 2020; accepted on October 15, 2020

Insects and pathogens can reduce the production of tomato [*Solanum lycopersicum* (Solanaceae)] fruits for industrial processing. Insect may cause direct or indirect damage, including the

transmission of virus from a few hours after tomato seedlings transplanting in the field (Pratissoli *et al.*, 2015). *Frankliniella* spp. (Thysanoptera: Thripidae) transmit virus to tomato

plants, causing the Tomato Spotted Wilt Virus (TSWV) disease. Fourteen, of the 1710 recognized species of the Thripidae family, were described as virus-vectors with estimated losses near

US\$1.4 billion in one decade in the United States of America (Riley *et al.*, 2011). *Frankliniella schultzei* is adapted to tomato plantations in the tropics where it became a key pest in Brazil (Pratisoli *et al.*, 2015). Resistance cases to insecticides by thrips (Gao *et al.*, 2012) indicate the need of evaluating new control methods to manage these pests.

Goiás state is one of the Brazilian leaders in the industrial processing and field production of tomatoes due, among other factors, the water availability for irrigation practices (Camargo Filho & Camargo, 2017). The great majority of the tomato crops for industrial processing in Brazil are irrigated by central pivot, and not by drip irrigation which has a less impact on air humidity because moisture is directly disposed to soil layers. The air humidity influences the efficiency of entomopathogenic fungi (Gatarayihá *et al.*, 2010). The microbial control of virus vectors in commercial tomato crops is still inexpressive (Assunção *et al.*, 2013), but entomopathogenic fungi may be an important tool to manage thrips (Wu *et al.*, 2014).

The entomopathogenic fungus *Beauveria bassiana* has been used to control sucking insects, such as Thripidae species, with selectivity to natural enemies (Wu *et al.*, 2014). The hypothesis tested was that plant resistance inducers, such as K_2SiO_3 , act synergistically with entomopathogenic fungi. This synergism has been demonstrated through different interactions, including the breakdown of lipid barriers in the insect cuticle by the K_2SiO_3 , facilitating the fungi penetration and colonization of the insect body (Storm *et al.*, 2016). The synergism between the fungus *B. bassiana* and K_2SiO_3 increased the mortality of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), in mono and dicotyledonous plants (Gatarayihá *et al.*, 2010), and thermotolerance and pathogenicity of fungus spores (Kim *et al.*, 2014).

The objective of this research was to evaluate the control efficiency (CE%) against the thrips *Frankliniella schultzei* with *Beauveria bassiana* (Bb), the

resistance inducer potassium silicate (KSil) and the chemical insecticide profenophos + cypermethrin (PC), isolated or in binary mixtures.

MATERIAL AND METHODS

Experimental site and design

The experiment was carried out in a tomato commercial area in the municipality of Pires do Rio (altitude 758 m), southeastern Goiás state, Brazil. The Heinz (HJ Heinz Company) industrial tomato hybrid 9553 was planted spaced 0.29 m between plants and 0.60 m and 1.20 m between simultaneous planting lines. Tomato plants for industrial processing are conducted with two different spacings between planting lines, due to the requirements of the mechanized harvest.

The experiment was in a randomized block design with four replications. Each parcel had 16 tomato plants in a 10-meter long row. The border between treatments within the blocks was 2 m with plants that were not evaluated. The seedlings were transplanted semi-mechanically with a transplant machine (Ferrari, model FX) with four transplanted seedlings spaced by 1.2 m between rows and 36 tray capacity. Cover fertilization was applied at a dose of 30 g plant⁻¹ (for each application) with mineral fertilizer 12-06-12 (N-P-K) at 20 days after transplanting and with 12-00-12 (N-P-K) at 40, 60, 80 and 100 days after seedling transplanting. Irrigation was performed by central pivot. The air humidity values during the experimental trials were 78% (0 day after application, DAA), 62% (1 DAA), 75% (7 DAA), 77% (14 DAA) and 73% (21 DAA). Temperature values were 28°C (0 day after application, DAA), 27°C (1 DAA), 29°C (7 DAA), 27°C (14 DAA) and 28°C (21 DAA).

Products application and characteristics

The treatments were represented by the products *B. bassiana* (Bb), potassium silicate (KSil) and profenophos + cypermethrin (PC), alone or in the binary mixtures of Bb+KSil, PC+KSil and Bb+PC applied, only once, using

a 20 L manual sprayer on the tomato leaves, up to the point of run off (200 to 300 L ha⁻¹), at 15 days after the tomato seedlings transplantation. All applicators used the appropriate personal protective equipment (PPE) required by the Brazilian legislation. The Bb used was the BeauveControl® (Simbiose®, Cruz Alta-RS, Brazil) (MAPA Brazil registration number 3816) with the isolated IBCB 66 (2x10⁹ CFU/g of the product) (4 g/kg and 4% m/m) in a wettable powder formulation (WP) with toxicological classification IV (low toxicity) and environmental hazard class IV (not dangerous to the environment). The dose of the Bb (commercial product) was 2.5 g L⁻¹ of water with a solution volume of 200 L ha⁻¹. The KSil was the silicon foliar product of the company Solo Fértil SP Comercial Agrícola Ltda (São José do Rio Preto-SP, Brazil) (MAPA Brazil registration number 0944610000-9). The potassium silicate (K₂SiO₃), used in a 0.4 L 100 L⁻¹ of water, has 12% by weight of silicon (Si) and 15% of potassium (K₂O) with a solution volume of 200 L ha⁻¹. The Polytrin® (PC) (Syngenta Proteção de Cultivos Ltda, São Paulo, Brazil) has 40% profenophos (organophosphate insecticide/acaricide) and 4% cypermethrin (pyrethroid insecticide) in an emulsifiable concentrate formulation (EC) registered in the MAPA of Brazil with the number 009507. The toxicological classification of this product is III (moderately toxic) with environment hazard level II (very dangerous to the environment). The PC was applied at a dose of 0.75 L ha⁻¹ with a solution volume of 300 L ha⁻¹ according to the technical control recommendation for *F. schultzei* on tomato plants.

Thrips sampling

Thrips were counted at 0, 1, 7, 14 and 21 daa (days after application of the products) by beating the terminal shoots of the upper third of the tomato plants on white color plastic trays (30 x 40 cm). This sampling is representative, practical and commonly used to evaluate thrips populations on vegetables. Ten plants were evaluated per plot with a total of 960 samples during the experimental period. All individuals sampled in

the field were immediately stored in plastic containers (200 mL) with 70% alcohol (70°GL) and 30% water, and brought to the laboratory where morpho-species were individualized. The thrips were mounted in slides and sent to a taxonomist for identification.

Quantified parameter and statistical analysis

Control efficiency (CE%), per treatments and time intervals (days after application), were calculated using the Hedderson & Tilton (1955) formula: $CE\% = 100 \times [1 - (NLI - \text{number of thrips before application} \times NLI \text{ after the application} / NLI \text{ in the control after the application} \times NLI \text{ in the treatment before application})]$. NLI= number of living insects.

Data were plotted on BoxPlot type graphs to help identifying outliers which were removed. Normality of the data was verified by the Lilliefors adhesion test and complemented with the histogram obtained with the SAEG® software (Ribeiro Junior & Melo, 2008). The control efficiency (CE%) data did not follow a normal distribution and, therefore, were transformed into $\log(x + 1)$ which made the standard deviations of the samples proportional to their means. The coefficient of variation (CV) was used to indicate the accuracy of the transformation of the real data to $\log(x + 1)$. The transformation was considered valid when the CV of the transformed

data was lower than those of the real one (Reed *et al.*, 2002). The normality was checked again and confirmed after data transformation. The analysis of variance (ANOVA, one-way type) and the comparison between the means were performed with the data transformed, however, the results in the figures and tables are the original ones.

The CE% data was calculated after eliminating the outliers and transforming the original result data before submitting them to ANOVA and the Tukey mean test at 5% significance level. Polynomial regression models were evaluated to find those with better fitting and by observing the adjusted coefficient of determination (R^2) for each model using SigmaPlot® software, version 11 (Systat Software Inc). The Mahalanobis cluster analysis was performed considering the residual covariance between the dependent and independent variables to classify the six treatments into groups as a function of the dependent variable (CE%) homogeneity and possible heterogeneity within each group using the SAEG® software.

RESULTS AND DISCUSSION

The CE% per treatment (individually or in binary combinations) differed at all time intervals evaluated (Table 1). At day one the Bb (*Beauveria bassiana*)

+ KSil (potassium silicate) had greater CE% for *F. schultzei* (95±5%) than the other treatments. The efficiency of the treatments PC (profenophos + cypermethrin), PC+KSil and Bb+PC, 75.92 to 81.58% was similar and (KSil) and Bb isolated showed the lowest CE% after 24 hours of the product application (35.25% and 21%, respectively). At seven days after application the CE% of treatment (PC) was the highest, 78.25%, and for Bb+KSil dropped from 95 to 62.25% with the highest efficiency losses between treatments (Table 1). The Bb and KSil had the lowest CE% values at seven days after the product application. At 14 daa, the efficiency of Bb+KSil was highest, 48±2.67% compared to all treatments. At the end of the experimental period (21 daa), the efficiency of the Bb and KSil was again the highest and Bb+KSil, the lowest.

Regression analysis of the CE% of the six treatments on 1, 7, 14 and 21 days after their application showed a reduction throughout the experimental period for all of them (Figure 1). The decrease of the CE% was linear for treatments T1 and T2 (Figure 1) and quadratic for T3, T4, T5 and T6 (Figures 1C, D, E and F, respectively). The results differed among treatments, with higher and lower CE% for T4 (Bb+KSil) and T1 (Bb) at all time intervals (Figure 1A, 1D). Regression equations, including adjusted R^2 , F and P values, and Standard Error of

Table 1. Control efficiency (CE%) (Mean±SE¹) against *Frankliniella schultzei* (Thysanoptera: Thripidae) on tomato plants for industrial processing from 1st to 21th day after application of the treatments isolated or in binary mixtures. Urutaí, IF Goiano, 2019.

| Treatment | Days after application (DAA) | | | |
|-----------|------------------------------|----------------|----------------|----------------|
| | 1 | 7 | 14 | 21 |
| Bb | 21.00 ± 1.29 d | 15.00 ± 1.08 d | 10.50 ± 0.64 d | 7.00 ± 0.21 d |
| PC | 81.58 ± 3.53 b | 78.25 ± 3.40 a | 17.00 ± 0.91 c | 10.25 ± 0.12 c |
| KSil | 35.25 ± 1.88 c | 21.25 ± 1.31 d | 11.50 ± 0.64 d | 8.00 ± 0.33 d |
| Bb+KSil | 95.00 ± 5.00 a | 62.25 ± 2.65 b | 48.00 ± 2.67 a | 41.50 ± 4.34 a |
| PC+KSil | 75.92 ± 4.28 b | 67.00 ± 3.62 b | 17.25 ± 1.88 c | 16.75 ± 3.81 c |
| Bb+PC | 79.50 ± 7.80 b | 53.75 ± 2.05 c | 22.75 ± 0.85 b | 20.50 ± 3.32 b |
| F | 45.43 | 130.54 | 86.73 | 78.03 |
| P | 0.02 | 0.02 | 0.02 | 0.01 |
| CV(%) | 13.48 | 9.07 | 14.07 | 12.21 |

¹Averages followed by the same letter, in each column, do not differ by the Tukey test at 5% significance. Bb = *Beauveria bassiana*; PC = profenophos + cypermethrin; KSil = potassium silicate.

Estimate (SEE) are presented: T1 (Bb): $21.97 - 1.08x + 0.01x^2$, adjusted $R^2=0.90$, $F= 68.65$, $P<0.0001$ and $SEE= 1.78$, T2 (PC): $91.18 - 4.13x$, adjusted $R^2=0.84$, $F= 81.14$, $P<0.0001$ and $SEE= 13.75$, T3 (KSil): $37.98 - 2.84x + 0.06x^2$, adjusted $R^2=0.95$, $F= 164.32$, $P<0.0001$ and $SEE= 2.33$, T4 (Bb+KSil): $100.02 - 6.16x + 0.16x^2$, adjusted $R^2= 0.91$, $F= 79.06$, $P<0.0001$ and $SEE= 6.54$, T5 (PC+KSil): $85.96 - 5.14x + 0.07x^2$, adjusted $R^2= 0.82$, $F= 36.69$, $P<0.0001$ and $SEE= 12.00$, and T6 (Bb + PC): $87.48 - 6.52x + 0.15x^2$, adjusted $R^2=0.89$, $F= 63.54$, $P<0.0001$ and $SEE= 8.54$. The analysis of hierarchical grouping, through the mean Mahalanobis distance, indicated that T4 treatment (Bb+KSil) differed from the others (Figure 2). The treatments T1 (Bb) and T2 (PC) formed a group and the T3, T5 and T6 another one. The KSil (T3) differed from the other treatments (T5 and T6) in this last group. Distance index from treatments T5 (PC+KSil) and T6 (Bb+PC) were smaller than the other ones, indicating the minor CE% differences (Figure 2).

The CE% of *F. schultzei* differed between treatments. Pest control recommendation in Goiás state is made

by signed contracts, between suppliers (farmers) and industry, prior to the planting (Assunção *et al.*, 2013) when the recommendation of highly efficient product with low environmental impact is necessary. In most cases, synthetic chemicals are recommended based on the assumption that they are more efficient, which may be not always true. Microbiological products (Bb) and even resistance inducers (KSil) are, normally, not recommended for tomato plant protection because their efficiency does not consider combined blends. The Integrated Pest Management (IPM) is a necessity for thrips control in tomato crops in Brazil and the synergism between products should be exploited to reduce the pressure on insect populations by the exclusive use of insecticides.

The low CE% of PC alone, mainly, at the end of the experimental period (21th daa) may be explained by the dissipation rates for the acaricide profenophos and the insecticide cypermethrin (in a range of seven days) of 98.4% and 73.5%, respectively, with a half-life of 1.35 and 4.11 days respectively (Nath *et al.*, 2005). Profenophos + cypermethrin is registered for *F. schultzei* control

in tomato crops in Brazil, with 6 applications per cycle with an interval between three (for high infestations) to seven days (for low infestations). The degradation of organophosphorus and mainly pyrethroid insecticides explains their low residual impact and higher frequency of applications for high thrips infestations (Nath *et al.*, 2005). The loss of CE% of the insecticide isolated in the municipality of Pires do Rio, over time, observed through a decreasing linear regression model, may be due, also, to the mean temperature of 27.6°C and relative humidity (RH) of 66% in the experimental period which are known to decreased efficiency of chemical insecticides, such as organophosphorus and pyrethroids (Javaid *et al.*, 2016).

The greater losses of CE% for the chemical PC mixed with the fungus Bb may be due to negative effects on germination and number of conidia of this fungus in mixture with certain groups of insecticides. Bb conidia germination was inhibited by 53.67%, 100.00% and 100.00% by the insecticides imidacloprid (neonicotinoid), lufenuron and dimethoate (100%) (the last two organophosphates) but it was not

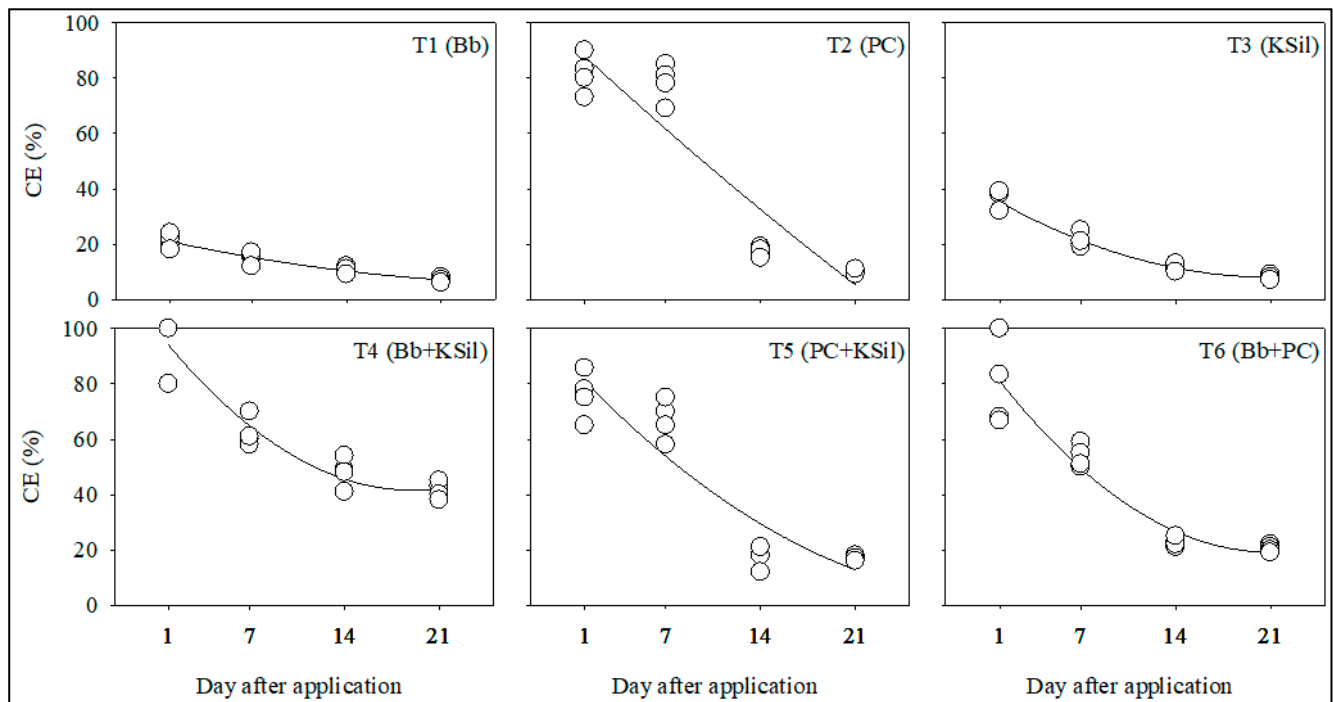


Figure 1. Regression analysis for the control efficiency (CE%) against *Frankliniella schultzei* (Thysanoptera: Thripidae) on 1, 7, 14 and 21 days after the application of the treatments isolated or in binary mixtures on tomato plants for industrial processing. Bb = *Beauveria bassiana*; PC = profenophos + cypermethrin; KSil = potassium silicate. Pires do Rio, IFGoiano, 2019.

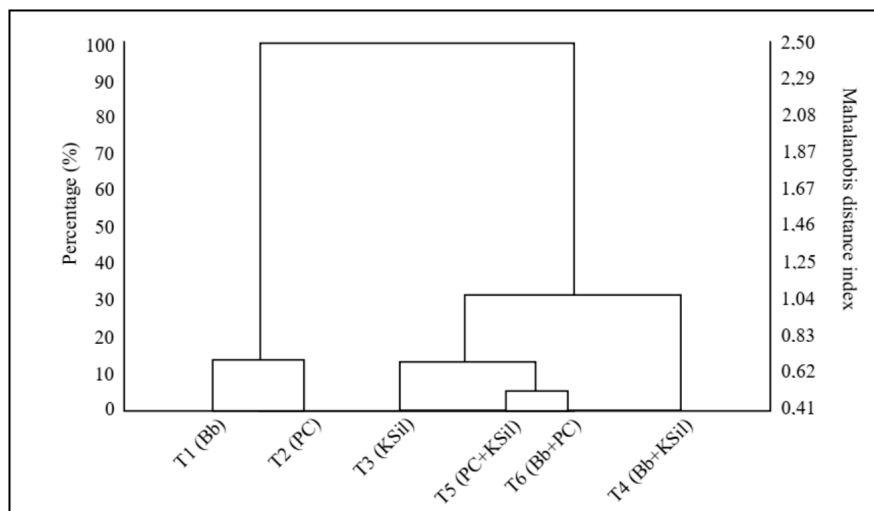


Figure 2. Dendrograms, based on the Mahalanobis distances, representing Control Efficiency (CE%) against *Frankliniella schultzei* (Thysanoptera: Thripidae) with the treatments applied alone or in a binary mixture on tomato plants for industrial processing. Bb = *Beauveria bassiana*; PC = profenophos + cypermethrin; KSil = potassium silicate. Pires do Rio, IFGoiano, 2019.

affected by the pyrethroid deltamethrin with half of its recommended dose (RD) (0.5 x RD) (Abidin *et al.*, 2017). The variation of the biological responses in the interaction between insecticides and fungi has been described through the behavioral selectivity of the target insect triggered by one of the agents in the mixture. This may occur through changes in the behavior of foraging and searching for food and also reflects in an annulment (James & Elzen, 2001) or, in contrast, potentiation of synergistic effect (Roditakis *et al.*, 2000). On the other hand, synergism in pest control among entomopathogenic fungi and insecticides was reported by Noskov *et al.* (2017). Due to the variation in results on this mixture between insecticides and fungi (used for biological pest control), new studies should better investigate possible cases of synergism or antagonism for thrips control in tomato plants.

The low control efficiency of KSil (isolated) against *F. schultzei* on the tomato plants is similar to that found for the mite *T. urticae* (Gatarayih *et al.*, 2010). Potassium silicate (KSil), or other compounds that have silicon (Si) in its formulation, are described as important physical and physiological resistance inducers against the presence of insects and diseases in plants, inducing plant protection side effects

(Reynolds *et al.*, 2016). Silicon is an essential element for certain plants, especially those of the families Poaceae, Equisetaceae and Cyperaceae (Currie & Perry, 2007), but tomato (Solanaceae) has limited absorption capacity for this element, which characterizes it as a non-accumulating plant (Mitani & Ma, 2005). This may explain why tomato plants are not excellent Si manipulators without activating its defense mechanisms against sucking insects, as occurs in the interaction between rice (*Oryza sativa*) (Poaceae) and *Nilaparvata lugens* (Hemiptera: Delphacidae) (Yang *et al.*, 2017). However, tomato plants seem to show better responses due to the presence of Si with higher yield parameters (Stamatakis *et al.*, 2003), water and nutritional balance (Wasti *et al.*, 2017), fruit quality and firmness (Stamatakis *et al.*, 2003) than defensive mechanisms against sucking insects.

The higher efficiency of Bb+KSil mixture may have been facilitated by the temperature (mean 27.8°C) and humidity (mean 73%) conditions during the experiment. *Beauveria bassiana* application is recommended when relative humidity is above 50%, according to technical recommendations from the supplier company. The synergistic effect of KSil on Bb under field conditions agrees with the compatibility of fungi

and silicate compound combinations to control insects and mites (Gatarayih *et al.*, 2010; Storm *et al.*, 2016). The powder containing Si are abrasive to the lipid surface layer of *Tribolium castaneum* (Coleoptera: Tenebrionidae) in stored grains (allowing a better fungal penetration) (Akbar *et al.*, 2004). This may not have occurred in our experiment, because a soluble (non-abrasive) source of Si was used. The synergism between Bb fungus and KSil increased mortality of the mite *T. urticae* by induced higher resistance in cucumber, eggplant, bean and maize plants and, therefore, increased susceptibility of this organism to fungus infections (Gatarayih *et al.*, 2010). However, as previously suggested, tomato plants do not present clear defensive responses mediated by Si against sucking insects. In addition, Si compounds may confer greater thermotolerance to fungal conidia, increasing their biological activity in the field. Bell & Hamal (1974) reported that *B. bassiana*, *Metarrhizium anisopliae* and *Metarrhizium rileyi* fungi spores maintained their virulence to insects after three years stored at -20°C in tubes containing silica gel crystals (a synthetic product made by the silicate reaction of sodium and sulfuric acid) compared to those without silica gel. Kim *et al.* (2014) have also confirmed higher thermotolerance mediated by Si compounds, through moisture retention, for the insect-parasitic fungus *Cordyceps fumosorosea*. The higher resistance to fungus applied under field conditions may have potentiated the degree of synergism between Bb+KSil observed in the present work in terms of CE%.

The synergistic effect of the KSil mixed with the parasite fungus *B. bassiana* indicate their potential to manage *F. schultzei* in tomato crops for industrial processing. We suggest, however, that our results should be extrapolated to real field conditions with caution, due to the peculiar experimental conditions observed, such as, for example, climatic conditions. In addition, the information can help to formulate more efficient mycoinsecticides adequate for the Integrated Pest Management as an alternative to reduce pesticide resistance

by sucking insects, such as *F. schultzei*.

ACKNOWLEDGEMENTS

To Mrs. Fernanda de Souza Ferreira that allowed the experimental procedures in her tomato production area. To Dr. Élisson F.B. Lima (Universidade Federal do Piauí, Floriano, Piauí state, Brazil) for thrips identification. To Dr. Miguel Michereff Filho (Embrapa Hortaliças) for reviewing preliminary versions of this work and for his criticism. To the National Council for Scientific and Technological Development (CNPq) for financial assistance. The Foundation for Research Support of Goiás State (FAPEG). To the PAPPE Program of the Instituto Federal Goiano for the research grant to the first author (Process n° 23216.000570/2017-26) and, finally, the Programa de Pós-Graduação em Proteção de Plantas (PPGPP) (IFGoiano, Campus Urutai).

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