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Effect of calcium polysulfide on Diaphorina citri (Hemiptera: Liviidae) and its primary parasitoids

Efecto del polisulfuro de calcio sobre Diaphorina citri (Hemiptera: Liviidae) y sus parasitoides primarios

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ABSTRACT

Huanglongbing (HLB) is a devastating disease with no current cure, affecting citrus plantations worldwide. Controlling its spread relies heavily on managing the Asian citrus psyllid (*Diaphorina citri*), the disease's primary vector. In response to the dependence on chemical insecticides, which can negatively impact biodiversity and induce pest resistance, this study evaluates calcium polysulfide as an alternative compatible with the parasitoids *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, specific biological control agents of *D. citri*. The research was conducted under laboratory conditions, testing six concentrations of calcium polysulfide across different developmental stages of the insects. Data distribution was reviewed using the Shapiro-Wilk normality test, and then an analysis of variance (ANOVA) with a significance level of $p < 0.05$, followed by Duncan's test for mean comparisons. Results demonstrated that concentrations of 0.24% and 0.31% significantly reduced the survival of *D. citri* nymphs and eggs, without interfering with the emergence of parasitoids in the mummy stage, due to the protective effect of the pest's cadaver (mummy) on the pupae. It was concluded that calcium polysulfide was not harmful to *T. radiata* and *D. aligarhensis* under exposure conditions typical of the mummy stage, and its detrimental effects on *D. citri* were restricted to specific developmental stages (egg and nymph), without affecting other stages evaluated. Therefore, its integration into integrated pest management (IPM) programs is feasible, provided that application timing minimizes exposure of more sensitive parasitoid stages (adults).

Keywords: Asian citrus psyllid; biological control; Huanglongbing; integrated pest management; pesticide selectivity; primary parasitoids

RESUMEN

El Huanglongbing (HLB) es una devastadora enfermedad que a la fecha no tiene cura y que afecta a plantaciones de cítricos en todo el mundo. Su control depende en gran medida del manejo del psílido asiático de los cítricos (*Diaphorina citri*), el principal vector de la enfermedad. En respuesta a la dependencia de los insecticidas químicos, que pueden afectar negativamente la biodiversidad e inducir resistencia en la plaga, este estudio evalúa el polisulfuro de calcio como una alternativa compatible con los parasitoides *Tamarixia radiata* y *Diaphorencyrtus aligarhensis*, agentes de control biológico específicos de *D. citri*. La investigación se llevó a cabo en condiciones de laboratorio, evaluando seis concentraciones de polisulfuro de calcio en diferentes etapas de desarrollo de los insectos. La distribución de los datos se verificó mediante la prueba de normalidad de Shapiro-Wilk y luego se aplicó un análisis de varianza (ANOVA) con un nivel de significancia de $p < 0.05$, seguido de la prueba de Duncan para la comparación de medias. Los resultados demostraron que las concentraciones de 0,24% y 0,31% redujeron significativamente la supervivencia de ninfas y huevos de *D. citri*, sin interferir en la emergencia de los parasitoides en la etapa de momia, debido al efecto protector del cadáver de la plaga (momia) sobre las pupas. Se concluyó que, el polisulfuro de calcio no resultó perjudicial *para T. radiata* y *D. aligarhensis* cuando la exposición se produjo durante la fase de momificación. Su efecto perjudicial sobre *D. citri* se limitó a fases específicas del desarrollo (huevo y ninfa), sin afectar a otras fases evaluadas. Por lo tanto, su integración en programas de manejo integrado de plagas (MIP) es factible, siempre que el momento de aplicación minimice la exposición de las fases más sensibles de los parasitoides (adultos).

Palabras clave: control biológico; Huanglongbing; manejo integrado de plagas; pesticida selectivo; parasitoides primarios; psílido asiático.

INTRODUCTION

Huanglongbing (HLB) is the most devastating disease affecting citrus crops worldwide. Within just fifteen months of detection in a plantation, it can reduce production by up to 2.4 tons per hectare (Flores-Sánchez *et al*., 2015). In the Americas, HLB is primarily caused by the bacteria *Candidatus* Liberibacter asiaticus (CLas) and *Candidatus* Liberibacter americanus (CLam). It affects the phloem, impairs root functionality, and severely impacts tree productivity (Cavichioli *et al*., 2024). Since there is currently no cure for the disease, the most effective way to control its spread is through vector management (Alonso-Hernández *et al*., 2024).

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the primary vector of CLas (Wan *et al*., 2024), with this variant being the most distributed in Colombia. Globally, a wide variety of organisms that naturally control *D. citri* populations have been identified, among which the parasitoids *Tamarixia radiata* (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Hymenoptera: Encyrtidae) stand out for their specificity to this species (Hoddle *et al*., 2014; Vankosky & Hoddle, 2019; Wan *et al*., 2024). However, the rapid spread of the disease has led to intensive use of chemical products to control vector populations, which can generate pest resistance and reduce biodiversity in plantations (Bale *et al*., 2008).

In response to this issue, there has been a growing search for alternatives to chemical control, promoting more environmentally friendly approaches (Smaili, *et al*., 2020). Among these alternatives are mineral-based products, such as lime sulfur or calcium polysulfide, a compound obtained through the thermal treatment of sulfur and lime (Abbot, 1945). This product has demonstrated insecticidal, acaricidal, and fungicidal effects, and various studies suggest that at low concentrations, it may be less harmful to natural enemies compared to conventional chemical products (Montag *et al*., 2005; Venzon *et al*., 2007; Fajardo *et al*., 2013). Calcium polysulfide not only has a reduced and selective toxicity profile towards natural enemies but also aligns with the principles of integrated pest management (IPM), making it a promising alternative to reduce dependence on synthetic pesticides.

Given that effective control of the vector *D. citri* is crucial to prevent the spread of HLB (Yan *et al*., 2020), this study aimed to evaluate the compatibility of low-toxicity calcium polysulfide with the parasitoids *T. radiata* and *D. aligarhensis*, to enable their coexistence within an integrated pest management program.

MATERIALS AND METHODS

The study was conducted at the Research and Breeding Center for Natural Enemies of the University of Caldas (coordinates: 5.056553, -75.494959), using insects from the arthropod breeding facility at Granja Montelindo (coordinates: 5.075097, -75.672948) at the same university.

Transparent containers measuring 11.5 cm in length by 11 cm in diameter (30 oz capacity) were used, containing shoots of *Murraya paniculata* along with the specimens to be evaluated, arranged on a moistened foam base to ensure the turgidity of the material. Treatments were applied by direct spraying of calcium polysulfide (32°B). Subsequently, the containers were covered with muslin cloth to facilitate aeration and placed in a Thermo Scientific brand incubator at a temperature of 25 ± 2 °C and 60 ± 10% relative humidity. For the containers with parasitoids, a strip of filter paper with microdrops of honey was added to ensure feeding.

Six concentrations within the range of the median lethal concentration determined by Restrepo-García and Soto-Giraldo (2017) were evaluated under a completely randomized experimental design in a 6×3×2 factorial model (concentrations: 0% (control), 0.1%, 0.17%, 0.24%, and 0.31%; insect species: *D. citri*, *T. radiata*, and *D. aligarhensis*; developmental stages: nymphs and adults of *D. citri*, mummies and adults of the parasitoids). This resulted in a total of 36 treatments, each with 5 replicates and 5 insects per replicate. Additionally, each concentration was evaluated on the eggs of the psyllid. Data were subjected to the Shapiro-Wilk normality test, and subsequently, an analysis of variance (ANOVA) was performed with a significance level of $p < 0.05$, followed by a mean comparison using Duncan's test ($p < 0.05$) using the SPSS® version 20 software.

RESULTS

The survival rate of the insect species during the evaluation was affected by the different concentrations of calcium polysulfide, the exposure time, and the developmental stage, showing highly significant differences ($p < 0.05$) compared to the control (0%).

D. citri

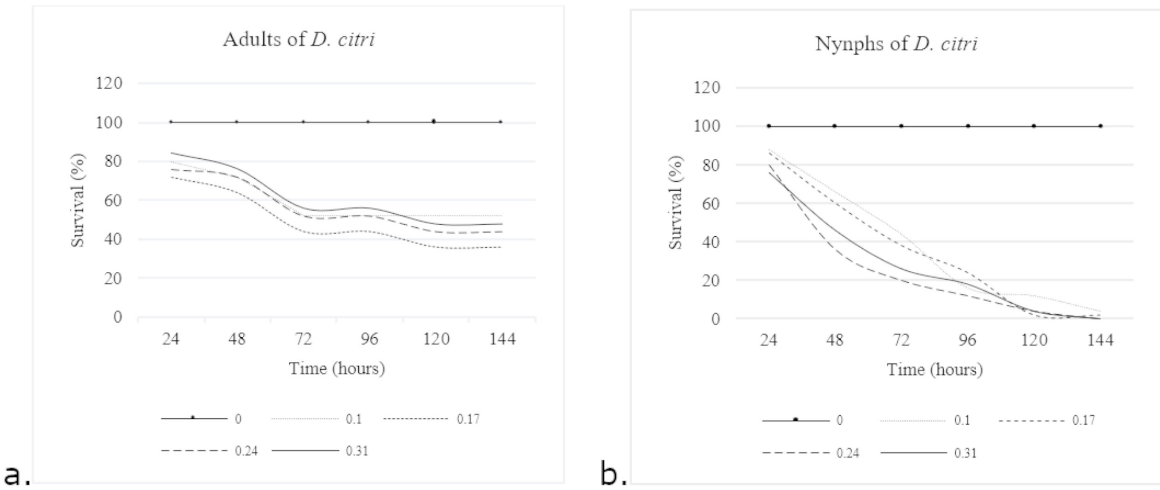
The survival rate of the psyllid decreased over time at all concentrations and all developmental stages, except in the control, where it remained constant at 100%. For adults, although a reduction in survival was observed at doses of 0.1% and higher, it was not as pronounced as in the nymphs. After 72 hours, the survival rate for concentrations of 0.24% and 0.31% stabilized around 44-48% (Figure 1a). On the other hand, a dose-dependent decrease in survival was observed in the nymphs, especially in the days following exposure as a sublethal effect. The most significant effect at this developmental stage was the hardening of the cuticle, which affected the molting process. Doses from 0.1% to 0.31% showed significant differences compared to the control starting at 48 hours, reaching the lowest survival values after 120 hours (4% to 0%) (Figure 1b).

T. radiata

The survival rate of the parasitoid at the adult stage showed a progressive decline from the first 24 hours after the application of calcium polysulfide. At a concentration of 0.1%, survival was 36% at 24 hours and dropped to 0% at 144 hours. At concentrations of 0.17% and 0.24%, survival was 24% and 16%, respectively, at 24 hours, reaching 0% at 120 hours in both cases. The highest concentration, 0.31%, reduced survival to 8% at 24 hours and to 0% at 96 hours (Figure 1c). Regarding the mummies, the percentage of parasitoid emergence was evaluated, and no significant differences were found between treatments ($p > 0.05$). This indicates that the product concentrations do not affect the immature stage of the parasitoid, as at the end of the observations, the number of emerged adults was equivalent to the number of mummies introduced into the experimental unit, and exit holes of the parasitoid were observed (Figure 1d).

D. aligarhensis

The response of this parasitoid was similar to that of *T. radiata*. The survival rate of *D. aligarhensis* adults was affected by the different concentrations of calcium polysulfide and exposure time. At a concentration of 0.1%, survival was 44% at 24 hours, dropping to 0% at 144 hours. At concentrations of 0.17% and 0.24%, initial survival was 28% and 20%, respectively, with values reducing to 0% at 120 and 144 hours. The highest concentration, 0.31%, resulted in survival of only 12% at 24 hours, reducing to 0% at 96 hours. For *D. aligarhensis* mummies, the analysis showed that the percentage of parasitoid emergence did not vary significantly between the different concentrations of calcium polysulfide ($p > 0.05$). This suggests that the immature stage of the parasitoid is not susceptible to the effects of the treatment, maintaining the viability of adult emergence without significant alterations.



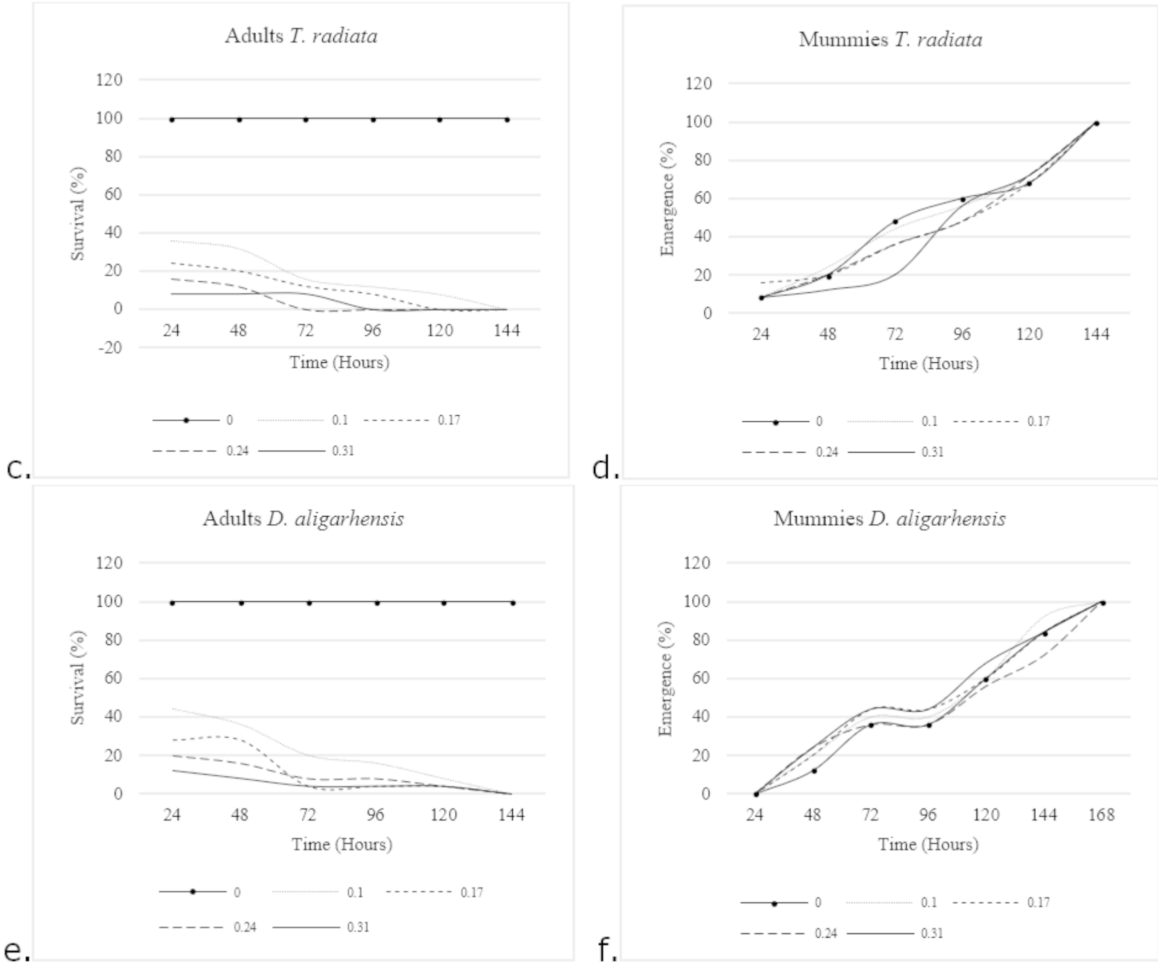


Figure 1. *Response of the different species to applications of calcium polysulfide. a. Adults of D. citri, b. Nymphs of D. citri, c. Adults of T. radiata, d. Mummies of T. radiata, e. Adults of D. aligarhensis, and f. Mummies of D. aligarhensis.*

DISCUSSION

The dose-dependent response observed in *D. citri*, particularly in the nymph and egg stages, highlights the potential of calcium polysulfide as a direct control agent. These findings agree with previous research by Restrepo-García and Soto-Giraldo (2017), who reported an LC_{50} of 0.38% for calcium polysulfide against *D. citri* nymphs. In the present study, concentrations of 0,24% and 0,31% significantly reduced survival, suggesting that these doses approach or exceed the median lethal concentration identified in earlier trials.

The effects observed in adult parasitoids confirm their susceptibility to the evaluated concentrations of calcium polysulfide. This finding contrasts with previous studies indicating that lime sulfur (calcium polysulfide), when applied at sublethal doses, was selective toward natural enemies (Dimetry *et al.*, 1993; Schmutterer, 1997; Castiglioni *et al.*, 2002; Soto *et al.*, 2011). On the other hand, the results also suggest that the cuticle of the *D. citri* cadaver (mummy) provides a protective barrier for parasitoids during their immature stage. Similar protective mechanisms were reported by Al-mazra'awi and Ateyyat (2009), who observed that mummies of *Bemisia tabaci* parasitized by *Eretmocerus mundus* offered effective coverage against applications of plant extracts.

Additionally, the evaluation of the product on *D. citri* eggs demonstrated an increase in mortality percentage correlated with the concentrations of calcium polysulfide, evidencing an ovicidal effect across all treatments. These results align with those reported by Ferrándiz (2015). Among the findings were: 1) dehydrated eggs, consistent with results obtained by Tsai and Liu (2000); 2) psyllid nymphs trapped in the chorion post-hatching, possibly due to the caustic action of the product (Ferrándiz, 2015); and 3) nymphs that, despite emerging, died within a few hours (Restrepo-García & Soto-Giraldo, 2017).

The results of this study indicate that, based on the concentrations evaluated, doses ranging from 0.24 to 0.31% could provide effective suppression of *D. citri* eggs and nymphs while maintaining parasitoid emergence under field conditions, provided that application frequency and timing are adjusted to local biological cycles. Applications should be carefully scheduled to coincide with periods when parasitoids remain protected within the host mummy, thereby minimizing exposure of free-living adults. Therefore, field evaluations are required to validate these findings, establish safe application intervals and frequencies, and ensure the sustainable integration of calcium polysulfide into citrus integrated pest management (IPM) programs.

CONCLUSIONS

It is recommended to avoid the simultaneous use of adult *T. radiata*, *D. aligarhensis*, and calcium polysulfide. However, the application of the highest dose of the product (0.31%) is advisable when *D. citri* is in the nymphal stage, and the parasitoids are in the mummy stage, as the product does not affect their emergence, due to the protection provided by the *D. citri* cadaver (mummy) to the parasitoid pupa.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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