# Effects of phosphine and plant extracts on flower thrips mortality and the quality of cut flowers

## Efectos de la fosfina y extractos de plantas sobre la mortalidad de trips de las flores y la calidad de flores de corte

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## ABSTRACT

Flower thrips represent a complex of significant quarantine species affecting the cut flower market in Colombia. The aim of this research was to evaluate postharvest treatments using phosphine in conjunction with a plant extract for thrips control and quality control of five cut flower species. Eight treatments were used: six employed a commercial dose of magnesium phosphide as a source of phosphine, one used a double dose and a control group without phosphine application. The first six treatments followed a bi-factorial structure, incorporating three exposure times and the addition of a chili-garlic extract. Thrips control efficacy was evaluated using the Schneider-Orelli index based on field-collected samples. Postharvest quality assessments were conducted on roses, carnations, alstroemerias, chrysanthemums, and hydrangeas over an 18-d period following treatment application. Differences in efficacy were observed between the two locations (the blocking factor). Discrepancies in phosphine efficacy may be related to the variations in populations collected from different crops and locations, both in the departments of Cundinamarca and Antioquia. Variations in magnesium phosphide concentration, both at the commercial dose of 3.4 g $m^{-3}$ and double this amount (2X) did not produce significant differences in treatment efficacy or flower quality. The use of chili pepper and garlic extract applied by nebulization at 3°C combined with phosphine application also did not significantly affect thrips mortality efficacy. The factor most influencing efficacy improvement was exposure time, as longer time periods led to better thrips control. Furthermore, we found that longer exposure times did not affect visual quality or vase life, assessed through changes in color, physiopathies, and chlorophyll content.


**Key words:** postharvest phytosanitary treatment, chili garlic extract, quarantine pest, rose, chrysanthemum, hydrangea, carnation, alstroemeria.


## RESUMEN

Los "trips de las flores" representan un complejo de importantes especies cuarentenarias que afectan al mercado de la flor cortada en Colombia. El objetivo de este estudio fue evaluar tratamientos poscosecha con fosfina y un extracto vegetal para el control de los trips y la calidad de cinco especies de flor cortada. Se aplicaron ocho tratamientos: seis de ellos empleando una dosis comercial de fosfuro de magnesio como fuente de fosfina, uno utilizando una dosis doble, y un grupo de control sin aplicación de fosfina. Los seis primeros tratamientos siguieron una estructura bifactorial, incorporando tres tiempos de exposición y la adición de un extracto de ajo-ají. La eficacia del control de trips se evaluó mediante el índice de Schneider-Orelli basado en individuos colectados en campo. Se realizaron evaluaciones de la calidad poscosecha en rosas, claveles, astromelias, crisantemos y hortensias durante un periodo de 18 d tras la aplicación del tratamiento. Se encontraron diferencias en la eficacia entre las dos ubicaciones (el factor de bloqueo). Las discrepancias en la eficacia de la fosfina podrían estar relacionadas con las variaciones en las poblaciones recolectadas de diferentes cultivos y ubicaciones, tanto en el departamento de Cundinamarca como en el departamento de Antioquia. Las variaciones en la concentración de fosfuro de magnesio, tanto a la dosis comercial de 3.4 g $m^{-3}$ como al doble de esta (2X), no mostraron diferencias significativas en la eficacia del tratamiento ni en la calidad de las flores. El uso del extracto a base de ajo y ají, aplicado por nebulización a 3°C combinado con la aplicación de fosfina, tampoco afectó significativamente la eficacia del control de los trips. El factor que más influyó en la mejora de la eficacia fue el tiempo de exposición, ya que períodos más largos condujeron a un mejor control de los trips. Además, se encontró que los tiempos de exposición más prolongados no afectaron la calidad visual ni la vida útil en el jarrón, evaluada mediante la variabilidad en los cambios en el color, el contenido de clorofila y la presencia de fisiopatias.


**Palabras clave:** tratamiento fitosanitario poscosecha, extracto de ajo ají, plaga cuarentenaria, rosa, crisantemo, hortensia, clavel, astromelia.

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## Introduction

Quarantine pests are organisms that are absent or under active control that pose an economic or environmental risk; and, therefore, their entry into Colombia as pests is controlled (IPPC, 2021). For cut flower exports in Colombia, shipment interceptions because of the presence of quarantine pests are one of the main limitations (ICA, 2018), where thrips, lepidoptera, aphids, and mites stand out as the most significant pests (Brownbridge & Buitenhuis, 2019). For the cut flower market, the term "flower thrips" represents a group of quarantined insect species such as *Frankliniella panamensis* and *Thrips palmi* (Teulon *et al*., 2014). Thrips are an important pest because of their polyphagous character and high reproductive rate (Taylor, 1994) and because they generate direct damage mainly on flower buds and young leaves, tissues, and consumption of cellular content (Herrick *et al*., 2021). In addition, they cause indirect damage because they are potential virus vectors and because of their quarantined nature (Jones, 2005). MacLeod *et al*. (2004) estimate economic losses of £16.9 to £19.6 million over a decade due to *T. palmi* in chrysanthemums in southern England. These losses include reduced yield and quality, extra research, plant health certification expenses, and export losses. They also note a substantial sixfold rise in pest control spending on a 4.8 ha greenhouse site (MacLeod *et al*., 2004).

Aspects such as pest tolerance, decrease in the number of available insecticides, and product market requirements favor the development or innovation of integrated pest management alternatives. Export crops require pest management in the field, complemented with post-harvest strategies. Post-harvest phytosanitary treatments (PPT) include physical methods such as ultrasound, temperature, controlled atmospheres, irradiation (Nicholas & Follett, 2018) or chemicals such as essential oils (Kostyukovsky & Shaaya, 2001), ethyl formate, and fumigants such as methyl bromide ($CH_3Br$) and phosphine ($PH_3$) (Sirohi *et al*., 2021). PPTs are an additional phase in the production chain and their improper use can affect quality, especially those that involve high doses (Liu, 2011). For this reason, it is important to ensure that PPTs do not interfere with the cold chain or generate negative physiological effects such as maturation in living tissues (Fields & White, 2002; Su-Kim *et al*., 2016).

Methyl bromide has been used as an effective postharvest pest treatment because of its broad spectrum and versatility. However, since 1987, methyl bromide was listed as harmful to the ozone layer in the Montreal protocol, where the United Nations Environment Program (UNEP) proposed a gradual reduction of its use from 1997 to critical levels in 2005 (UNEP, 2020). Phosphine is a versatile, economical, and effective alternative to methyl bromide (Arora *et al*., 2021) since it acts at the respiratory level, inhibiting the transport of electrons in the mitochondrial complex IV, limiting energy production; this is why it is highly toxic to aerobic organisms such as insects (Karunaratne *et al*., 1997; Zhang *et al*., 2013). Phosphine gas is characterized by rapid diffusion that allows it to reach all spaces in the treated space, has low permeability and low absorption in plant material that makes it relatively harmless, and requires prolonged exposure times to be highly effective (Zhang *et al*., 2013). Because the sensitivity of agricultural products to fumigants varies with the species, it is important to determine aspects such as exposure times, doses and forms of application that control pests without affecting the quality of the agricultural product (Liu & Liu, 2014).

Phosphine gas ($PH_3$) is generated by hydrolysis produced by contact of ambient humidity with the solid state of phosphides (Carvajal Oviedo *et al*., 2014). The market has two forms of phosphides used for the generation of phosphine: the first is aluminum phosphide (AlP) used mainly in stored products and the second is magnesium phosphide ($Mg_3P_2$) used in fresh products and grains (Anasac Colombia, 2018; Restrepo-Giraldo, 2019). Commercial presentations of aluminum phosphide contain ammonium carbamate ($NH_2CO_3NH_4$) in its formulation; this reacts exothermically to generate ammonia ($NH_3$) and carbon dioxide ($CO_2$). The former is phytotoxic in fresh vegetables, and the latter accelerates ripening by stimulating the production of ethylene; its use is limited in fresh products (Anasac Colombia, 2018; Restrepo-Giraldo, 2019). Magnesium phosphide may or may not contain ammonium carbamate; however, the flower market mostly uses the formulation without ammonium carbamate that only generates phosphine gas and an innocuous solid residue (Huber-Valiño, 2019; Nath *et al*., 2011). Phosphine treatments under conditions of refrigeration require longer exposure times than those carried out at room temperature because the volatilization rate decreases with temperature (Hole *et al*., 1976; Liu, 2011; Zhang *et al*., 2013). However, the exportation of fresh products such as flowers are time-limited in terms of dispatch times and refrigerated storage spaces; so, it is necessary to identify the species to be controlled and the treatment times for control to minimize time.

Species such as garlic (*Allium sativum*) and chili (*Capsicum* sp.) are a source of plant extracts used as shock insecticides at low incidences or as pest repellents (Lema-Jami, 2011). Garlic hydrolate has antimicrobial and antifungal

properties because of its ability to limit oxygen absorption by pathogens, affecting their growth and stress responses that cause damage to plants (Juárez-Segovia *et al*., 2019). The chili pepper is characterized by its high content of the alkaloid capsaicin that affects the functioning of chemoreceptors and nociceptors in arthropods, thus, generating signals and stimuli of potentially harmful irritation in the tissue that affect behavior and defense mechanisms and induce stress and evasion reactions (Li *et al*., 2020). Capsaicin is not phytotoxic to ornamental plants and has proven effective in controlling pests such *as Plutella xylostella* in vegetables, reducing their population by up to 55.94% without compromising cabbage production quality (Baidoo & Mochiah, 2016). Given the alterations that some plant extracts produce in insects, their use is proposed as an innocuous alternative that favors the efficacy of phosphine treatments. This research evaluated the effect of treatments with phosphine and its complement with a chili-garlic extract as a postharvest management strategy for controlling flower thrips and quality in the main species of cut flowers for export from Colombia.

## Materials and methods

### Location

Two trials were established in principle locations for the production and export of cut flowers in Colombia in 2021: The first is eastern Antioquia in the municipality of Rionegro (Antioquia) (6°8'19.42" N, 75°24'53.02" W, altitude 2100 m a.s.l.), and the second in the Bogotá savannah in the municipality of Mosquera (Cundinamarca) (4°41'47.72" N, 74°12'42.54" W, altitude 2550 m a.s.l.). For the Antioquia experiment, daytime temperature conditions were 18.99°C ± 2.16°C and nighttime temperature was 15.96°C ± 1.01°C, while for Cundinamarca, daytime temperature was 15.96°C ± 1.01°C and night time temperature was 15.3°C ± 0.68°C. Relative humidity in both experiments was similar, approximately an average of 79%.

### Plant species and insect collect

The five most important flower species in the Colombian export market were included in the experiment. In Antioquia, chrysanthemums (*Chrysanthemum* sp. var. Maisy) and hydrangeas (*Hydrangea macrophylla*. var. White) were used, while, in Cundinamarca, roses (*Rosa* sp. var. Snow Bliss), carnations (*Dianthus caryophyllus* var. Grand Slam), and alstroemerias (*Alstroemeria* sp. var. Himalaya) were used. The flowers were harvested 24 h before the application of the treatments and were subjected to a traditional post-harvest process for export based on Asocolflores (2010) and Fischer and Flórez (1998). The number of stems per bouquet was adjusted according to the species, with 10 stems for chrysanthemum, 3 stems for hydrangea, and 12 stems for rose, carnation, and alstroemeria.

Based on the duration of the developmental stages of *Frankliniella* at a temperature of 25°C-30°C, thrips were directly collected from plants in the field. This suggests that nymphal, preoviposition, and adult longevity stages of both females and males were found on leaves and flowers, establishing them as pests capable of causing damage to the crop (Solís, 2016). Thus, thrips were collected from infested flowers the day before the experiment was begun; they were counted and confined in plastic cups (16 oz or 473 ml) with a lid and a cotton plug to ensure gas exchange. A flower or inflorescence of the species was collected as a source of food and shelter (Kim *et al*., 2016).

### Experiment design and treatments

Eight treatments were established based on the dose of magnesium phosphide, the use of the plant extract, and the time of exposure to the treatments (Tab. 1). The first six treatments had a bifactorial structure where the factors were the use or lack of the plant extract and the second factor was exposure time. Additionally, according to the Andean standard for pesticide registration (ICA & ANDI, 2016), two control treatments were included: the first without phosphine (control 0X), and the second with a double dose (control 2X), both using a treatment time (exposure time) of 24 h. Magnesium phosphide (Fumicel Placa®, Anasac, Colombia) was used as a phosphine source with a commercial dose of 3.4 g $m^{-3}$. The plant extract (with a concentration of 54.2% garlic + 43.4% chili pepper extract, Capsialil SL®, Ecoflora Agro, Medellin, Colombia; a commercial dose of 1.0 ml $L^{-1}$ $H_2O$) was applied with thermal fogging with a portable device powered with butane gas (KB100, Hyundai, Seoul, South Korea) at a rate of 20 ml $m^{-3}$.

**TABLE 1.** Postharvest phytosanitary treatments evaluated, consisting of different doses of magnesium phosphide, their combination with a chili garlic extract, and the exposure time.

| Treatment | Magnesium phosphide dose (g $m^{-3}$) | Plant extract (20 ml $m^{-3}$) | Exposure time (h) |
|---|---|---|---|
| 1 | 3.4 | No | 12 |
| 2 | 3.4 | No | 18 |
| 3 | 3.4 | No | 24 |
| 4 | 3.4 | Yes | 12 |
| 5 | 3.4 | Yes | 18 |
| 6 | 3.4 | Yes | 24 |
| 7 | 0.0 (control 0X) | No | 24 |
| 8 | 6.8 (control 2X) | No | 24 |

### Treatment applications

The applications were carried out under refrigeration conditions in cold rooms (3°C ± 1°C), in which 200-L metal barrels with lids were used as treatment chambers. We used twenty-four barrels, corresponding to the eight treatments with three replicates each. Each barrel contained a glass with 14 thrips to ensure efficacy evaluation, along with two bouquets of flowers per species used for quality assessment. We applied the magnesium phosphide from a cup attached to the wall of each drum. In treatments involving the use of plant extracts, we applied the magnesium phosphide using a thermo-fogger. After the application of the extract and/or phosphide, we immediately sealed each drum. During the treatment period, we monitored for phosphine leaks from the barrels using a portable meter (PAC-8000, Dräguer, Germany). We quantified the concentration of phosphine using colorimetric tubes based on silver salts (Detia-Degesch®, Laudenbach, Germany). We carried out two evaluations for each barrel: the first 7 h after starting the treatments (initial measurement) and the second at the end of the exposure times (final measurement) that were used to estimate the average concentration. The barrels corresponding to each treatment were uncovered and ventilated for 15 min according to their exposure time, *i.e.*, zero day after treatment (DAT). We removed the flowers from the barrels and stored them in boxes for the simulated trip period, where the same refrigeration conditions were maintained.

### Environmental variables

During the treatment period, the simulated trip, and the vase quality tests, we monitored the environmental temperature and relative humidity with dataloggers (ELMA DT-171, Elma Instruments, Ryttermarken, Denmark). The average temperature inside the cold rooms was set at 3°C, but it varied between locations, being 2.49°C ±1.40°C for Antioquia and 3.08°C ± 0.50°C for Cundinamarca.

### Treatment's efficacy

We evaluated the mortality of thrips at 2 DAT using the count of dead individuals within each vessel. Those individuals that did not show movement after stimulation via repeated contact with a brush under incandescent light at 30 cm were deemed to be dead (Liu, 2011; Zhang *et al.*, 2015). We, further, inspected the thrips using a digital microscope at 500x (WiFi Digital Microscope, STPCTOU, China). We used the count of living and dead individuals to estimate the percentage of mortality as the quotient between the number of dead individuals with respect to the total number for each vessel. With these mortality values, we determined the percentage of efficacy with the Schneider-Orelli formula (Ciba-Geigy, 1981; ICA & ANDI, 2016) and Equation 1.

$$\text{Efficacy (\%)} \quad \frac{(b - k)}{(100 - k)} \times 100 \qquad (1)$$

Where b represented the average mortality percentage for the three replicates of the 0x control (Tab. 1), and k represented the percentage of mortality in each of the replicate of the treatment.

### Flower vase quality

We carried out the vase quality evaluations after the simulated travel period occurred under refrigeration conditions in cold rooms (3°C ± 1°C) for 7 d. For each species, there was a total of 9 branches, corresponding to the experimental units. The evaluations were carried out in rooms within the farms, under ambient conditions and under shade. We placed each bouquet in a glass vase to which we added 1.5 L of water from the aqueduct with a pH of 6.3 and an electrical conductivity of 1.8 μS $cm^{-1}$ and this was replaced every 2 d until the end of the trial to prevent the accumulation of microorganisms and avoid the use of disinfectants in the solution. The quality variables we evaluated were chlorophyll, color, and the presence of physiopathies that were assessed at 12 d, 14 d, 16 d, and 18 d after treatment (DAT) (Caldua-Pohl, 2015; Figueroa *et al.*, 2005; López *et al.*, 2008; Mosqueda-Lazcares *et al.*, 2011).

We assessed the chlorophyll content in the upper third of the foliage using a portable chlorophyllometer (SPAD-502, Konica Minolta, Osaka, Japan) on three randomly selected leaves in duplicate. We measured petal color (CieLab) on three random points of the petal surface in duplicate using a colorimeter (CR-400, Minolta Camera Co., Osaka, Japan). Additionally, at 18 DAT, we recorded the incidence and average severity of physiopathies and diseases described in Table 2, typically assessed in similar experiments.

**TABLE 2.** Description of the assessed physiopathies in postharvest cut flower species.

| Common name of a physiopathy | Description |
|---|---|
| Bluing | Discoloration in the petals due to loss of pigments or copigmentation with flavonoids or related compounds (Arévalo-Hernández, 2011; Chaudhry, 1997; Cho *et al*., 2020; Halevy & Mayak, 2011). |
| Dehydration | Loss of generalized turgor of the tissues (Arévalo-Hernández, 2011). |
| No opening | Absence of the natural opening of the flower, also known as rest or dormancy (Arévalo-Hernández, 2011). |
| Petal fall | Premature loss of petals (Chaudhry, 1997; Mosqueda-Lazcares *et al*., 2011). |
| Neck bent or nodding | Loss of turgor and firmness in the portion of the stem immediately below the flower head that causes its curvature (Mosqueda-Lazcares *et al*., 2011). |
| Oxidation or necrosis in petal and foliage | Presence of oxidation or tissue necrosis in petals or foliage (Chaudhry, 1997; Cho *et al*., 2020). |
| End of vase life | When more than 50% of the flower petals in each bouquet fell, they began to turn gray (discoloration) or showed necrosis or wilting (Chaudhry, 1997; Mosqueda-Lazcares *et al*., 2011). |

### Statistical analysis

Analyses were performed with the statistical software R (R Core team, Ver. 1.2.1235, 2020). The average phosphine concentration variable was analyzed with analysis of variance (ANOVA) for all treatments that included phosphine (excluding treatment 7) and taking the locality as a blocking factor. For efficacy, two analyses were performed: the first was an analysis of covariance (ANCOVA) using the "agricolae" library (De Mendiburu, 2021), taking the concentration of phosphine as a covariable, the locality as the first factor and the treatments as the second factor. The second type of analysis for efficacy was limited to treatments 1 to 6 and was performed using ANOVA due to its bifactorial structure, taking the location as the blocking factor, the use of the plant extract as the first factor, and the exposure time as the second factor. The differences between groups were estimated using the Tukey test using the "agricolae" library (De Mendiburu, 2021).

For the evaluation of chlorophyll and color, given its longitudinal nature, we used a longitudinal analysis of variance with the "nparLD" library (Noguchi *et al*., 2012), taking the applied treatment as the first factor and the time in days after treatment as the second factor. In the different analyses of variance, in the absence of interactions, we analyzed the simple factors independently (Montgomery, 2017). Differences between levels were interpreted from error bars (Cumming *et al*., 2007). We evaluated the presence of patho-physiologies descriptively using the count of affected branches and the number of stems with the pathophysiology for each one. We produced graphics using the "ggplot2" library (Gómez-Rubio, 2017).

## Results and discussion

There were differences in the average temperature inside the cold rooms between localities even though they were programmed the same (3°C), where Antioquia was lower than Cundinamarca by approximately half a degree although the latter had less variation. These variations responded to aspects of design and the operation of the cold rooms; but, in both cases, they described a traditional scenario of refrigeration in the post-harvest processes of cut flowers. Temperature plays a fundamental role in the conservation of cut flowers since it influences the production of ethylene and maturation (Gómez Rubio *et al*., 2017), and postharvest treatments with phosphine can affect performance (Hole *et al*., 1976; Liu, 2011). During the evaluation of quality in a vase, there were differences in the average temperature between evaluation localities resulting from altitude, with lower values in Cundinamarca than in Antioquia. However, this did not influence the tests since different flower species were evaluated between localities.

The difference between day and night temperatures was similar between the test locations and the difference was not greater than half a degree (Antioquia: 0.33°C, Cundinamarca: 0.44°C). Relative air humidity was higher inside the cold rooms than outside during the vase tests. Differences in relative humidity between environments were identified, and the Cundinamarca trial was higher than in Antioquia although the latter had greater variability. At the environmental level, the relative humidity was similar between environments and between day and night.

We found statistically significant differences for the average concentration of phosphine (*P value*: 0.085), given by treatment 8 (2X control) (287.50 mg $L^{-1}$) that differed from treatments 1 (195.83 mg $L^{-1}$), 4 (179.16 mg $L^{-1}$) and 6 (170.83 mg $L^{-1}$) (Tab. 3). Between treatments 1 to 6, the difference in phosphine concentration was not statistically different. For the locality factor, there were significant differences (*P* value: <0.001): the concentration used in Antioquia (176.19 mg $L^{-1}$) was lower than in Cundinamarca (241.66 mg $L^{-1}$).

**TABLE 3.** Average phosphine concentration in parts per million (mg $L^{-1}$) for the eight treatments, the two locations and general average.

| Phosphine concentration per treatment | | | |
|---|---|---|---|
| **Group** | **At 7 h** | **Final** | **Average** |
| Treatment 1 | 183.33 ± 47.14 | 208.33 ± 34.35 | 195.83 ± 33.59 b |
| Treatment 2 | 183.33 ± 68.71 | 241.66 ± 53.35 | 212.50 ± 59.07 ab |
| Treatment 3 | 183.33 ± 23.57 | 233.33 ± 143.37 | 208.33 ± 75.92 ab |
| Treatment 4 | 183.33 ± 23.57 | 175.00 ± 25.00 | 179.16 ± 22.43 b |
| Treatment 5 | 166.66 ± 37.26 | 250.00 ± 81.64 | 208.33 ± 55.27 ab |
| Treatment 6 | 150.00 ± 28.86 | 191.66 ± 67.18 | 170.83 ± 44.29 b |
| Treatment 8 (2X) | 266.66 ± 106.71 | 308.33 ± 97.53 | 287.50 ± 89.84 a |
| **Phosphine concentration per location** | | | |
| Group | At 7 h | Final | Average |
| Antioquia | 169.04 ± 66.32 | 183.33 ± 35.63 | 176.19 ± 43.96 B |
| Cundinamarca | 207.14 ± 58.32 | 276.19 ± 105.35 | 241.66 ± 72.10 A |
| General | 188.09 ± 65.29 | 229.76 ± 91.32 | 208.92 ± 68.09 |

Values represent the mean plus/minus the standard deviation for the group. The letter at the end denotes significant differences according to Tukey's test with a 95% confidence estimated for the mean concentration, where lowercase letters describe differences between treatments and uppercase between test sites. The concentration inside the treatment 7 barrel (control 0X) was measured using a portable PAC-8000 meter, without detecting the presence of phosphine in it. Treatment 1: 3.4 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of chili garlic extract (CGE) and 12 h of exposure time (ET). Treatment 2: 3.4 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 18 h of ET. Treatment 3: 3.4 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 24 h of ET. Treatment 4: 3.4 g $m^{-3}$ of $Mg_3P_2$, 20 ml $m^{-3}$ of CGE and 12 h of ET. Treatment 5: 3.4 g $m^{-3}$ of $Mg_3P_2$, 20 ml $m^{-3}$ of CGE and 18 h of ET. Treatment 6: 3.4 g $m^{-3}$ of $Mg_3P_2$, 20 ml $m^{-3}$ of CGE and 24 h of ET. Treatment 7 (control 0X): 0 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 0 h of ET. Treatment 8 (control 2X): 6.8 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 24 h of ET.

The differences between treatments with the same dose of magnesium phosphide may respond to variations in the dosage that is carried out manually just before sealing the barrels or to the low volume of these or microleakage of phosphine gas, typical of fumigation.

For average phosphine concentration, no statistically significant effect was found on the response variables (Tab. 4). The locality factor showed significant differences with respect to the thrips mortality variable, where Cundinamarca had a higher mean mortality than Antioquia; however, the efficacy did not show differences between localities. Finally, the treatment factor did affect the response in mortality and efficacy (Tab. 4).

**TABLE 4.** Results of the analysis of covariance (ANCOVA) for the efficacy variable by the Schneider-Orelli index.

| Source of variation | F Value | P Value |
|---|---|---|
| Average concentration (covariable) | 0.578 | 0.453 |
| Location (factor 1) | 10.663 | 0.003 |
| Treatment (factor 2) | 383.197 | <0.001 |
| Location x treatment (interaction) | 1.024 | 0.435 |

The 0X control (treatment 7) showed the lowest efficacy (2.5%) and described the expected mortality of the population resulting from factors external to the trial (Fig. 1). The efficacy results obtained for the 0X control showed that the insects were adequate for use in the study. The use of insects collected in the field was accepted because it reflected the natural genetic diversity that is desirable if there are enough individuals; however, this can lead to broad experimental limits and mask small differences (Mouratidis *et al*., 2022). The breeding of individuals is the most used method for phytosanitary research (Heather & Hallman, 2008) since it allows control over the stages, uniformity in the species, and a high number of individuals that results in greater strength for statistical tests. In some species, the use of individuals raised in the laboratory for phytosanitary tests is not recommended unless their susceptibility does not differ from wild individuals (Badenes-Pérez & López-Pérez, 2018).

For the treatments with phosphine, the 2X control (treatment 8) was characterized by an efficacy of 97.5%, similar to treatments 3 and 6 (98.6%), all characterized by a long exposure time. A trend of increased efficacy was identified when the exposure time was greater, while the treatments with the lowest exposure times, such as treatment 1 and 4, showed lower efficacy values (92.5% and 94.0%) (Fig. 1). The average concentration of phosphine did not significantly affect the efficacy of the treatments even though concentrations between 170 mg $L^{-1}$ (treatment 6) and 285 mg $L^{-1}$ (treatment 8) were obtained, equivalent to 61% more phosphine (Tab. 5). The commercial dose of 3.4 g $m^{-3}$ of magnesium phosphide (treatments 1 to 6) was adequate to control thrips but it was higher than that used in other thrips studies: *F. occidentalis*, where the maximum

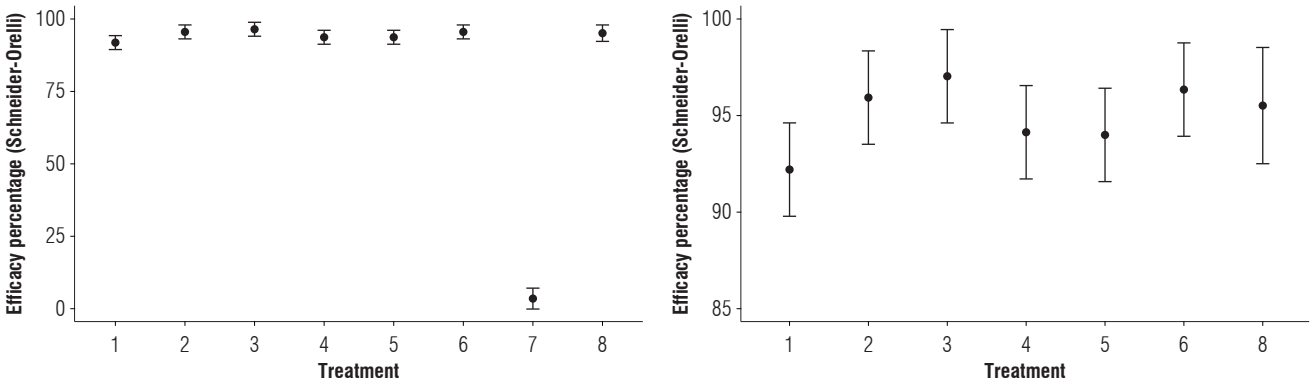


**FIGURE 1.** Efficacy response of postharvest phytosanitary treatments applied to flower thrips. On the left, the effect for all treatments is shown, while on the right, the treatment was ignored, and the visualization scale was enlarged to show differences between the other groups. The vertical bars represent the 95% confidence intervals for the model. Total data number: 48. Treatment 1: 3.4 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of chili garlic extract (CGE) and 12 h of exposure time (ET). Treatment 2: 3.4 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 18 h of ET. Treatment 3: 3.4 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 24 h of ET. Treatment 4: 3.4 g $m^{-3}$ of $Mg_3P_2$, 20 ml $m^{-3}$ of CGE and 12 h of ET. Treatment 5: 3.4 g $m^{-3}$ of $Mg_3P_2$, 20 ml $m^{-3}$ of CGE and 18 h of ET. Treatment 6: 3.4 g $m^{-3}$ of $Mg_3P_2$, 20 ml $m^{-3}$ of CGE and 24 h of ET. Treatment 7 (control 0X): 0 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 0 h of ET. Treatment 8 (control 2X): 6.8 g $m^{-3}$ of $Mg_3P_2$, 0 ml $m^{-3}$ of CGE and 24 h of ET.

mortality is reached with 1.52 g $m^{-3}$, 24 h of exposure and 2°C storage (Liu, 2008) or that of Zhang *et al*. (2015), who reports 100% mortality with a dose of 1.66 g $m^{-3}$ and 16 h of exposure.

For the factorial analysis of efficacy (Tab. 5), we saw no interaction effects in any of the variables, so we analyzed the simple factors separately. For a blocking factor, described as the trial location, there was a statistically significant effect on efficacy. The exposure time factor showed differences for both mortality and efficacy (Tab. 6). Finally, the plant extract use factor did not affect the response in thrips mortality and efficacy (Tab. 5).

**TABLE 5.** Results of the analysis of variance (ANOVA) for the efficacy variable with the Schneider-Orelli index.

| Source of variation | *F Value* | *P Value* |
|---|---|---|
| Trial location (block) | 17.979 | <0.001 |
| Exposure time (factor 1) | 5.145 | 0.012 |
| Plant extract (factor 2) | 0.489 | 0.489 |
| Exposure time x Extract (interaction) | 1.141 | 0.253 |

The efficacy presented statistically significant differences for the locality factor (Fig. 2) that was lower in Antioquia (93.22%) than Cundinamarca (97.10%). The events that occurred may be attributed to the variation in the population of thrips in the different plant species, a phenomenon influenced by environmental conditions like temperature, rainfall, and the phenological stage of the crops. A high level of genetic diversity is known for the species *F. occidentalis* through ribosomal analysis, indicating the presence of a population structure associated with both geographic region and host plants (Turcios Palomo, 2013). Therefore, we suggest that fluctuations in the effectiveness of phosphine may be attributable to differences in the populations collected from different crops and locations, both in Cundinamarca and Antioquia. This highlights the importance of considering regional and host-specific factors in the management of thrips populations in agricultural settings.

For the exposure time factor (Fig. 2), there were significant differences, with 12 h being the exposure time with the least efficacy (93.22%), followed by 18h (95.47%) and finally 24 h with the greatest efficacy (96.78%). However, only the 12 h and 24 h treatments differed significantly. These results supported the fact that under refrigeration conditions, exposure times should be increased to ensure the absorption of phosphine by insects; Liu (2008) and Liu (2011) find a maximum control effect with 18 h of exposure (250 mg $L^{-1}$ of phosphine). Also, Karunaratne *et al.* (1997) report that higher treatment temperatures can decrease exposure times in adult *Heliothrips haemorrhoidalis*. However, in postharvest cut flowers, temperature is a difficult factor to alter since thrips are highly sensitive to temperature (Gómez *et al*., 2017) so it would be necessary to understand previous studies and the limits of maximum temperatures that do not affect the quality or durability of the product.

For the factor related to the use of extract at its commercial dose, we found no statistically significant differences between the two groups (Fig. 2). We anticipated that the extract would enhance insect metabolism due to its well-known repellent properties, but this effect was not

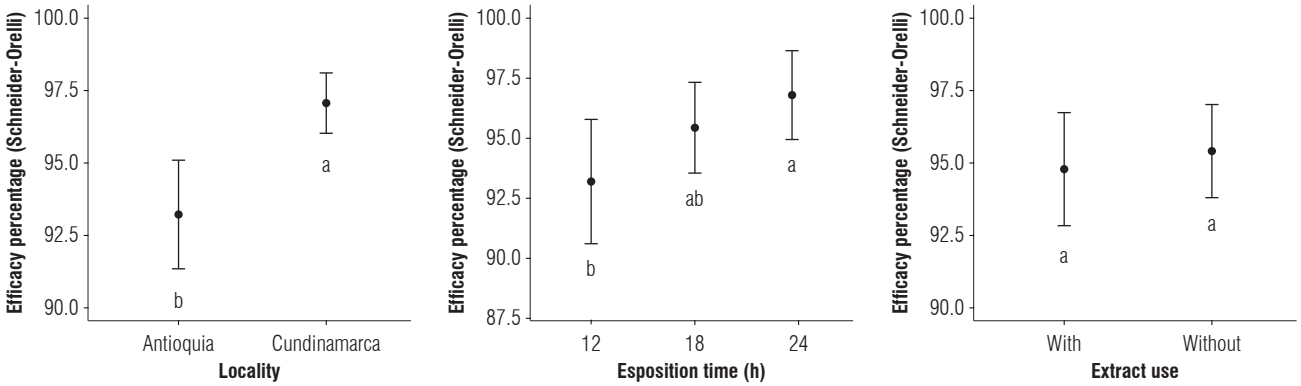


**FIGURE 2.** Efficacy response of simple factors to postharvest phytosanitary treatments applied to flower thrips. The left figure represents the effect from the blocking factor given by the test location. The central figure represents the effect of the exposure time factor. The figure on the right represents the effect of the factor associated with the use of the plant extract. The vertical bars represent the 95% confidence intervals for the model. Different letters denote statistically significant differences according to Tukey's test (confidence 95.0%). Total data number:48.

significant. This suggests that the presence of the plant extract did not induce sufficient stress in the thrips to elevate their metabolic rate and facilitate the absorption of phosphine. On the other hand, the low influence of the extract on efficacy could be due to the loss of effectiveness of the garlic extract, possibly due to the volatility of the compound called allicin after 2 h (Trabuco *et al*., 2015). Furthermore, the low temperature (3°C) may constrain thrips activity, potentially diminishing extract absorption and, consequently, its impact (Baidoo & Mochiah, 2016; Kazem & El-Shereif, 2010).

The results of quality variables at environmental temperature mostly showed an effect due to sampling time, attributed to changes from maturation to the flower's senescence. However, further research on changes during vase life due to fumigation may be necessary to fully understand the senescence effect of the implemented varieties.

We observed significant differences in chrysanthemums for the a* component due to treatment and time interaction. Hydrangeas exhibited changes in the b* component as a result of treatment effects. Roses showed effects from the interaction for the SPAD variable and treatment factors for both a* and b* components. Carnations displayed changes in the SPAD variable due to interactions. We found no significant differences related to the applied treatments for alstroemerias (Tab. 6).

The senescence of cut flowers depends on different factors, so there is no standard treatment for all species that favors floral longevity (Gómez *et al*., 2017). Senescence is genetically regulated and expressed through the signaling of the hormone ethylene that is the main cause of maturation and senescence in fresh agricultural products. In flowers, the time it takes for the marked symptoms of wilting to appear defines floral longevity (Ciba-Geigy, 1981; Gómez *et al*., 2017; Juárez-Hernández *et al*., 2008; Van-Altvorst & Bovy, 1995). The decrease in chlorophyll content is associated with maturation that derives from an increase in the synthesis of ethylene and chlorophyllase enzymes (Balaguera-López *et al*., 2014). The relative content of chlorophyll (in SPAD units) in roses and carnations showed significant effects from the interaction of the treatments with the quality evaluation time, where treatments 7 and 8 (controls 0X and 2X) showed lower values than the other treatments. A clear trend was not observed, and the variation between the values was low; this means that the treatments do not show negative effects on the quality of the color in the leaves determined by the chlorophylls (Zhang *et al*., 2013).

The color of the petals is one of the most important quality attributes in cut flowers; they manifest by pigments such as carotenoids and flavonoids, the main pigments found in flowers (Tanaka *et al*., 2009). In cut flowers, color changes result from senescence of the flower, which increases with time after removal from the plant, when the flower deteriorates and loses its commercial value (Castellanos *et al*., 2016; Gómez *et al*., 2017). Although white flowers were used for most of the species evaluated in this research, changes in color components over time were identified in almost all of them in the postharvest quality evaluation.

In this trial, minimal responses were observed in color components from the effect of the treatments. The L* component, which describes the changes in luminosity, did not show differences, indicating that the darkening of the petals from browning and tissue oxidation are not related

**TABLE 6.** Results of the non-parametric longitudinal analysis (ANOVA type), for relative chlorophyll content in SPAD units of leaves and for components of the CieLab color space in petals in the cut flower species.

| Species | Variable | TTO | DAT | TTO x DAT | CV |
|---|---|---|---|---|---|
| Chrysanthemum | SPAD | 0.100 | 0.000 | 0.118 | 4.31 |
| Chrysanthemum | L | 0.086 | 0.000 | 0.454 | 2.38 |
| Chrysanthemum | a* | 0.000 | 0.000 | 0.000 | 34.60 |
| Chrysanthemum | b* | 0.059 | 0.000 | 0.060 | 18.80 |
| Hydrangea | SPAD | 0.142 | 0.000 | 0.103 | 7.92 |
| Hydrangea | L | 0.097 | 0.000 | 0.905 | 2.75 |
| Hydrangea | a* | 0.138 | 0.000 | 0.715 | 28.22 |
| Hydrangea | b* | 0.067 | 0.000 | 0.140 | 16.58 |
| Rose | SPAD | 0.026 | 0.754 | 0.007 | 6.41 |
| Rose | L | 0.836 | 0.004 | 0.089 | 1.40 |
| Rose | a* | 0.072 | 0.000 | 0.195 | 15.08 |
| Rose | b* | 0.000 | 0.000 | 0.177 | 6.71 |
| Carnation | SPAD | 0.135 | 0.000 | 0.001 | 3.19 |
| Carnation | L | 0.891 | 0.000 | 0.911 | 17.18 |
| Carnation | a* | 0.890 | 0.001 | 0.873 | 14.96 |
| Carnation | b* | 0.973 | 0.175 | 0.509 | 25.46 |
| Alstroemeria | SPAD | 0.691 | 0.166 | 0.565 | 6.57 |
| Alstroemeria | L | 0.419 | 0.000 | 0.934 | 8.14 |
| Alstroemeria | a* | 0.924 | 0.226 | 0.895 | 55.71 |
| Alstroemeria | b* | 0.681 | 0.000 | 0.112 | 71.50 |

TTO: treatment; DAT: days after treatment; TTO x DAT: interaction of treatments and days after treatment; CV: coefficient of variation in percentage.

to the treatments evaluated. For component a*, describing the color change between red (positive) and green (negative), the treatments only generated effects in chrysanthemums, probably associated with enzymatic degradation of chlorophyll in chrysanthemums in the initial stages of the evaluation; in the later evaluation days (16 and 18 DAT) this difference is not evident (Pathare *et al*., 2013). For this reason, we inferred that the variations observed respond to experimental error at the time of evaluating the color or variations in the flowers and not to the senescence of the flowers. The b* component, which describes changes between yellow (positive) and blue (negative), showed differences in the rose treatments because of lower values in treatments 6 and 7. This variation did not imply a significant effect in color since changes were imperceptible to the eye as long as they remained close to light colors of the yellow region (b* close to 15), characteristic of white varieties such as Snow Bliss roses (Pathare *et al*., 2013).

Some of the physiopathies were identified in roses and hydrangeas, since both species are characterized by their postharvest susceptibility. In roses, we detected signs of necrosis and reddish discoloration in the petals, possibly due to the oxidation of floral tissues, influenced by environmental conditions such as low temperatures, high light radiation, and deficiencies in calcium and boron (Cabrera *et al*., 2007). Lu *et al*. (2009) found that 52% of the variation in anthocyanin content in petals of different cultivars of *Ipomoea purpurea* is related to temperature and UV radiation. Similarly, previous studies suggest that the genetic predisposition of the cultivar and low temperatures are determinants in the blackening of petals in red roses (Zieslin, 1968). In hydrangeas, necrosis was observed at the stem edge, possibly associated with soil pH. Brouillard (1988) indicates that soil pH can affect flower color through the physical interaction of electrons in pigments. Additionally, hydrangeas grown in soils with a pH of 5.5 exhibit blue flowers, whereas at pH 6.0 they are pink. The aluminum available at low pH accumulates in the petals, forming complexes with anthocyanins that produce the bluish color, and together with low temperatures, can induce tissue necrosis (Quintana *et al.,* 2007).

Regarding the use of the 2X dose, we observed no changes or effects due to the presence of physiopathies. Kim *et al*. (2016) finds no statistically significant effects of phytotoxicity in roses, lilies, and chrysanthemums treated with doses of magnesium phosphide at 2.0 and 4.0 g $m^{-3}$ for 24 h and

refrigeration at 8°C. However, it is important to consider for future evaluations that there are reports of phytotoxicity in cut flowers following treatments with phosphine (Zhang *et al*., 2013). Those authors observe effects up to 8 d after the application of treatments in 14 cultivars of cut flowers, with an increase in damage indices in some cultivars as exposure time and plant senescence increases.

## Conclusion

The treatments evaluated in this study proved to be effective in controlling flower thrips, with exposure time being the predominant factor over variables such as fluctuations in phosphine concentration and the use of double the commercial dose as well as the inclusion of plant extracts. The simultaneous use of garlic and chili pepper plant extract showed no significant influence on efficacy when applied in conjunction with phosphine. However, given that the storage temperature during treatments and the simulated travel period was 3°C for 7 d, it is plausible that the molecule mechanism of action may be affected by alterations and volatilities after a couple of hours and under low temperatures. To obtain a more precise understanding of the plant extract effects, it would be pertinent to conduct evaluations at different application times, encompassing each of the different varieties implemented in the trial.

Since phosphine treatments had no significant impact on the quality of flower species and their efficacy was over 97% at exposure times of 24 h, the use of phosphine as a tool for postharvest phytosanitary management in cut flowers is proposed. However, further evaluation across multiple cultivars and other species is recommended to corroborate and establish guidelines on its efficacy in flower thrips mortality.

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### Conflict of interest statement

The authors declare that there are no conflicts of interests regarding the publication of this article.

### Author´s contributions

PLP: conceptualization, investigation, data collect, statistical analysis, writing of original draft, visualization, and writing, review & editing. SBO: investigation, data collect, writing of original draft, and writing, review & editing. AH: supervision, project administration, writing, review & editing. All authors reviewed the final version of the manuscript.

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