ORIGINAL PAPER

Assessment of biorational insecticides to control thrips colonizing lettuce in greenhouse

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ABSTRACT

Thrips is one of the key lettuce pests that reduce yield and quality by direct damage caused mainly by piercing-sucking feeding and indirect damage by virus transmission. Conventional management of thrips is based on a few active ingredients of synthetic insecticides at the risk of generating resistance and environmental disruption. Currently, a new generation of insecticides of botanical origin and other natural derivatives are available on the market. The aim of this study was to evaluate the efficacy of biorational insecticides against thrips and their impact on natural enemies and lettuce production in a greenhouse. The assays were carried out in a greenhouse at INTA Concordia, Argentina during the autumn of 2022 and 2023. Three biorational insecticides were tested by contact: the positive control, azadirachtina (Neemazal® 1,2% EC, 0,4%), potassium oleate (Hydralene® AGK 48% SC, 2%), orange essential oil (OEO 10% SC, 0,3%), and additionally, water was included as a negative control. Applications began when thrips populations reached the economic injury threshold according to lettuce phenology. Thrips population and their natural enemies were inspected weekly on plants and after the first application also on yellow and blue sticky traps. Lettuce yield and losses were evaluated for each treatment at harvest. Neemazal® was the most effective bioinsecticide to reduce thrips infestation (42-53%), followed by Hydralene® (27-37%). No evident effect was obtained with the experimental OEO (0-19%). The reduction of the thrips infestation was directly related to their direct damage and inversely related to the crop losses (5-11%). No significant effects on natural enemies captured by sticky traps were observed, with the exception of ladybugs in the OEO treatment. Mode of action and the possibilities to include bioinsecticides in an IPM program are discussed.

Keywords: biopesticides; Thysanoptera; leafy vegetables.

RESUMEN

Los trips constituyen una de las plagas clave de la lechuga que pueden afectar su rendimiento y calidad por daño directo causado principalmente por su aparato raspador-suctor y daño indirecto por ser transmisores de virus. El manejo convencional de la plaga se basa en el uso de pocos principios activos con el riesgo de generar resistencia y disrupciones ambientales. Una nueva generación de insecticidas de origen botánico y otros derivados naturales están disponibles en el mercado. El objetivo fue evaluar la eficacia de insecticidas biorracionales sobre trips, su impacto sobre sus enemigos naturales y la producción de lechuga en invernadero. Los ensayos se realizaron en un invernadero en INTA Concordia, Argentina durante el otoño de 2022 y 2023. Se evaluaron tres insecticidas biorracionales por contacto: el control positivo, azadirachtina (Neemazal®1,2% EC, 0,4%), oleato de potasio (Hydralene®AGK 48% SC, 2%), aceite esencial de naranja (AEN 10% SC, 0,3%) y agua como control negativo. Las aplicaciones comenzaron cuando la población de trips alcanzó el umbral de daño económico de acuerdo con el estado fenológico de la lechuga. La población de trips y sus enemigos naturales fue monitoreada semanalmente sobre plantas y con trampas pegajosas amarillas y azules. Para cada tratamiento se evaluó el rendimiento y las pérdidas a cosecha. Neemazal® fue el más

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efectivo para reducir la infestación de trips (42-53%), seguido de Hydralene® (27-37%). No se obtuvo un efecto evidente con el AEN (0-19%). La reducción de la infestación de trips estuvo directamente relacionada con el daño directo e inversamente a las pérdidas a cosecha (5-11%). No se observaron efectos significativos sobre los enemigos naturales sobre trampas pegajosas, a excepción de los coccinélidos en el tratamiento con AEN. Se discute el modo de acción y las posibilidades de incluir bioinsecticidas en un programa de MIP.

Palabras clave: *insecticidas bioracionales; Thysanoptera; hortalizas de hoja.*

INTRODUCTION

Lettuce is one of the most important vegetable crops worldwide in terms of its production and consumption (FAOSTAT, 2024). In Argentina, annual lettuce production reaches approximately 300,000 tons which cover a sustained demand throughout the year (Secretaría de Agricultura, Ganadería y Pesca, 2023). Lettuce production is carried out in the field, in greenhouses and more recently in soilless, such as hydroponic or aquaponic cropping systems (Jordan *et al*., 2018). In all lettuce cropping systems, key pests such as aphids and Thysanoptera belonging to the family Thripidae, commonly known as thrips, find a suitable environment to increase their populations quickly and produce outbreaks during their short cycle. Thrips are small, soft-bodied insects which damage lettuce through oviposition and piercing-sucking feeding. Although this can lead to yield losses, the primary impact is on commercial quality due to aesthetic damage (Durr *et al*., 2022). In addition, they cause indirect damage by the transmission of Tospoviruses which cause significant economic losses (Marchiori *et al*., 2024; Rotenberg *et al*., 2015). Among lettuce pests, thrips become increasingly important due to rising temperatures and droughts as a result of climate change, which favour their outbreaks and increases the risk of food production losses in the future (Mukherjee *et al*., 2023).

In conventional lettuce cropping systems, thrips control relies on external inputs, such as synthetic pesticides, to reduce economic losses and meet market standards despite pest pressure. However, the efficacy of several insecticides was dramatically reduced due to the development of resistance as it was demonstrated in *Frankliniella occidentalis* (Pergande) (Maurastoni *et al*., 2023). In addition, a growing demand of healthy and safe food implies the reconversion of conventional lettuce production through the adoption of innovative technologies based on preventive measures according to those proposed by the Integrated Pest Management (IPM) (Collier, 2023; Barrière *et al*., 2014, 2015). However, when a curative measure is needed, conventional pesticides can be replaced or combined with biorational pest control agents (insecticides), which include active ingredients from plants (botanicals or plant extracts), microbes (microbials), minerals, and synthetic materials (Mahawer *et al*., 2024). Currently, a new generation of insecticides of botanical origin and other natural derivatives are available on the market. In recent decades, they have experienced worldwide growth, not only because of their efficiency in pest control, but also because they have a low environmental impact and are an effective means of achieving residue-free production in horticulture. Biorational insecticides can be integrated into other tools of IPM as biological control, however, possible side-effects on natural enemies should be considered (Acheuk *et al*., 2022; Ferraris, 2016; Messeling *et al*., 2014). From a social point of view, biorational insecticides serve as a compatible tool to mitigate conflicts in peri-urban areas close to the horticultural belts as a consequence of the application of conventional pesticides (Rosenstein *et al*., 2017). Nowadays, some biorational insecticides available on the markets are registered or in process of registration in Argentina (SENASA, 2024). Among botanical insecticides, neem oil extracted from *Azadirachtina indica* A. Juss seeds, is a broad-spectrum pesticide which can affect the main orders of insect pests, including Thysanoptera (Schmutterer and Singh, 1995; Schmutterer, 1990). Azadirachtin belongs to the family of limonoids, which make up the seed oil that has various effects on pests such as regulating insect growth, reducing their fecundity and fitness and acting as an antifeedant, anti-ovipositional and deterrent (Schmutterer, 1990). In this regard, Roychoudhury (2016) pointed out that the full mode of action of azadirachtina is still unknown. Among plant extracts, essential oils have an insecticidal effect on soft-bodied insects such as thrips, by interfering with their growth through disruption of the nervous system or physiological processes leading to insect death (Gondwal *et al*., 2024). Orange oil is an essential oil derived from Rutaceae family, which acts as a broad-spectrum bioinsecticide, rich in d-limonene, causing direct toxicity by contact and repellent action on aphids, mealybugs, and scales (Da Camara *et al*., 2015; Smith *et al*., 2018; Hollingsworth, 2005) however, limited information about their toxicity on thrips is available. Synthetic compounds such as soaps or fatty acids of potassium salts are considered biorational contact pesticides with a broad-spectrum and a low toxic effect to non-target organisms, causing death by dehydration because it produces the disruption of the cell membranes (Moss and Weed, 2001). The hypothesis of this study was therefore that the biorational insecticides would be able to control thrips on lettuce with low effect on their natural enemies. In line with this challenge, the aim of this study was to evaluate their efficacy on thrips and their impact on natural enemies and lettuce production in greenhouses.

MATERIALS AND METHODS

Site, experimental design and treatments

The study was carried out at the experimental station of the National Institute of Agricultural Technology (INTA) located in Concordia, Entre Ríos (Argentina) (latitude 31° 22'S, longitude 58° 07'W, altitude 44 m). The experiments were conducted in a greenhouse of 300 m^2 on lettuce cv. 'Vera' (Sakata®) during 2022 (from transplant April 1 to harvest May 11) and 2023 (from transplant April 4 to harvest May 17). The lettuce crop consisted of four beds of 1 m width and 28 m length (fig. 1, 2). In each bed, three rows of lettuce plants were transplanted when seedlings reached three leaves (V13 stage, BBCH scale) (Feller *et al*., 1995) and thinned to 25 cm within and between rows following a staggered pattern. Organic fertilizer (Nutrire®) was applied at the rate of 350 cm^3/10l in two times during the period from rosette to harvest lettuce stage.

Each lettuce bed was divided into four plots of 7 m^2 corresponding to a replication of each treatment (fig. 2). The treatments tested in this study were Neemazal® 1.2 EC (Wayne Chemical SRL, 60 U$S/l), a botanical insecticide composed of azadirachtin oil Neem, applied at the rate of 0,4% as a positive control. Hydralene AGK® 48% (Wassington SACIFEI, 9 U$D/l) based on potassium oleate was applied at a rate of 2%, and an experimental formulation based on orange essential oil 10% SC (Bioagro S.R.L., 16.5 U$S/l) was applied at a rate of 0,3%. In addition, a negative control (water) was included. Each treatment was replicated four times in a randomized block design (fig. 2). The biorational insecticides were applied using a symmetrical backpack sprayer (Guarany©) equipped with a cone nozzle. The criterion used for the first applications was based on the economic injury threshold (EIT), for thrips on lettuce proposed by the MAPAMA (2017) protocol, established in 1-3 thrips/plant from seedling to rosette stage. The EIT for lepidopteran larvae is set at 1 larva/20 plants and for aphids at 2 aphids/10 plants.

Effects of the treatments and meteorogical variables on thrips and their natural enemies on lettuce plants

The effect of treatments on thrips and the natural enemies observed was assessed by weekly visual inspection on 10 plants/plot (40 plants/treatment) situated in the middle of the plot of each treatment (fig. 2). In each survey, all leaves of each plant were inspected, and the number of thrips (adults and larvae) and their natural enemies were identified and registered. When visual identification was not possible in the greenhouse, a sample of 20 individuals was taken out to the laboratory and identified using a stereomicroscope Olympus SZ51.

Figure 1. General view of the experimental lettuce culture in the greenhouse at the INTA experimental station (Concordia, Entre Ríos, Argentina).

Throughout the lettuce cycle, meteorological data were recorded from the nearest meteorological station to the study area with the aim of assessing the effects of meteorological variables (maximum, mean and minimum temperatures (°C), relative humidity (%), and precipitation (mm)) on the population of thrips and their natural enemies.

Effects of treatments on thrips and the natural enemies on sticky traps

After the first application of the biorational insecticides, yellow and blue sticky traps (10x12 cm) were paired and placed at the center of the plot of each treatment (4 paired traps/treatment), leaving 7 m between contiguous traps on each bed and 2 m between the center of contiguous beds (fig. 2). Traps were placed above the lettuce canopy to estimate the impact of bioinsecticides on the attraction and capture of winged thrips and adults of natural enemies by these traps. Generalist predators (bugs, coccinellids, syrphids) which consider thrips as either primary or secondary prey are regarded as the natural enemies of this pest. It has been thoroughly studied that blue sticky traps are more efficient than yellow traps to attract thrips (Broughton and Harrison, 2012; Cho *et al*., 1995) highlighting the capture of *F.*

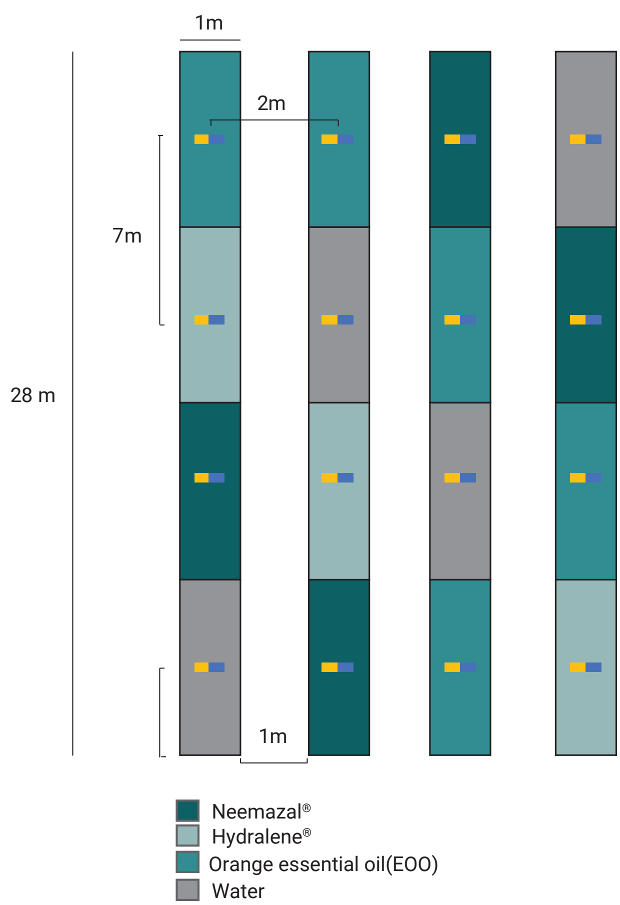

Figure 2. Schematic layout of the experimental lettuce crop, showing the plots of biorational insecticides tested in this study: Neemazal®, Hydralene®, an experimental formulation based on orange essential oil (OEO) and the negative control (water). ▬ indicates the position of the paired sticky traps (yellow and blue) located in the center of each plot.

occidentalis in greenhouse (Brødsgaard, 1989). However, yellow traps were added with the aim of complementing the capture of thrips on these traps and registering the capture of natural enemies attracted by this color (Shi *et al*., 2020). Traps were removed weekly before the application of biorational insecticides then covered with a polyethylene film and carried to the laboratory. After the application of each treatment, the traps were immediately replaced by new ones, which were set up as described above. Thrips and their natural enemies were counted on both sides of each trap using the same stereomicroscope.

Effects of treatments on lettuce yield

At harvest, 40 lettuce plants from each treatment were randomly selected from the center of each plot (10 plants/replicate/treatment). Plants were weighed individually to determine their gross weight (g). Then, the basal leaves damaged by thrips were removed to determine the marketable weight (g). Yield losses for each treatment were calculated as the difference between gross and marketable weight (g) and expressed as a percentage (%).

Statistical analysis

To determine the effects of each treatment on thrips populations, a generalized mixed model with a Poisson distribution was fitted, in which the response variable was the number of thrips detected per replicate (10 plants). Blocks, treatments, and year were considered fixed effects, while monitoring date was treated as a random effect. The inclusion of monitoring date as a random effect allowed the modeling of the dependence structure between observations from different monitoring sessions (Zuur *et al*., 2009). In addition, a fixed interaction effect between year and treatments was included. To evaluate the incidence of the treatments on the attraction and capture of thrips and their natural enemies by each trap, a generalized mixed model with a Poisson distribution was modeled with blocks, color traps, year, and treatments as fixed effects, and monitoring date as a random effect. Interaction terms between the fixed effects were included in the model and retained if they were significant, as occurred with treatments x traps. Gross weight (g), marketable weight (g), and yield losses (%) were analyzed using an analysis of variance (ANOVA). Meteorological variables (maximum, mean, minimum temperature (°C), and relative humidity (%) were included as covariates in the models to assess their influence on the thrips population. The fixed factors included in this analysis were block, treatments, year, and the interaction between year and treatments. The assumptions of normality and homoscedasticity were verified using the Shapiro-Wilk and the Levene test, respectively. Statistical analysis was performed using the functions “gls” and “glmer” from the “lme4” package in R (Bates *et al*., 2015; R3.6.3 Core Team, 2023; Tukey, 1949).

RESULTS

Effects of treatments and meteorological variables on thrips and their natural enemies

In both growing seasons, *Frankliniella* sp. and *Caliothrips phaseoli* were the Thripidae species identified as colonizing the lettuce plants. The populations of other key pests such as aphids and lepidopteran larvae remained below the EIT, so that no additional application of insecticides was necessary.

For thrips control, three applications of bioinsecticides were repeated weekly during both growing seasons. The thrips population reached the EIT (1-3 thrips/plant) at 14 and 21 days after transplanting in 2022 and 2023, respectively (fig. 3a, b). In 2022, after the third application (28 days after transplanting), thrips infestation decreased in all treatments until harvest (fig. 3a). After the first application, however, the increase in the number of thrips/plant was lower for Neemazal® and Hydralene® than for the OEO treatment (fig. 3a).

In 2023, thrips population continued to grow until harvest, despite the same number of bioinsecticides applications (fig. 3b). Notably, orange essential oil (OEO) produced the greatest increase in thrips per plant between the first application and harvest, compared to the control treatment (fig. 3b). Overall, thrips infesting lettuce showed significant differences between years ($F_{1,180}$= 521,7, p≤0,01) and treatments ($F_{3,180}$= 296,5, p<0,01). In addition, the interaction between both factors (year x treatment) was significant ($F_{3,180}$= 33,3, p≤0,01) indicating that the efficacy of the treatments on the pest was different between years.

Throughout the lettuce cycle, there were minor variations in the meteorological data between the study years (table 1). Temperature was slightly higher in 2023 at transplanting and from the rosette stage to harvest, while relative humidity was higher in 2022 than in 2023 during most of the growing season (fig. 3a, b). Temperature and relative humidity showed no significant differences in explaining the variability of thrips populations when included as covariates in the fitted model. Throughout the lettuce cycle, a total of 136 mm of precipitation was recorded in 2022 and 69,5 mm in 2023. The distribution of these precipitation amounts was observed to be over a period of 9 and 5 days in the respective years.

Neemazal® demonstrated the most significant impact on thrips infestation throughout the entire lettuce cycle, achieving an average of 4,3 ± 0,4 in 2022 and 8,2 ± 0,6 in 2023 thrips/plant. This reduction in thrips population on lettuce was 53% and 42%, respectively, compared to the control. Among all treatments, OEO registered the lowest effect on the pest in both years, reaching in 2022 an equal mean number of thrips/plant (9,2 ± 0,7) compared to the control. In 2023 the mean number of thrips/plant was 11,13 ± 0,9, representing a 19% reduction in thrips infestation compared to the control (fig. 4).

The effectiveness of Hydralene® showed a significant difference compared to the control in both years, reducing the mean number of thrips/plant between 27% (2022) to 37% (2023) (fig. 4). A significant difference in toxicity was observed between Neemazal® and Hydralene® at low thrips density/plant, as occurred in 2022, while this effect was not observed in 2023 at higher thrips infestations (fig. 4). In both years, only *Orius insidiosus* (Hemiptera: Anthocoridae) and spiders were the natural enemies observed on the plants. However, their abundance was low, and their presence was sporadic across all treatments; therefore, no data were analyzed.

Effects of the treatments on thrips and predators observed on sticky traps

As observed for thrips infestation on lettuce, differences in thrips density on traps were registered between both growing seasons (2022-2023) ($F_{1,204}$= 18,2 p≤ 0,01). The population

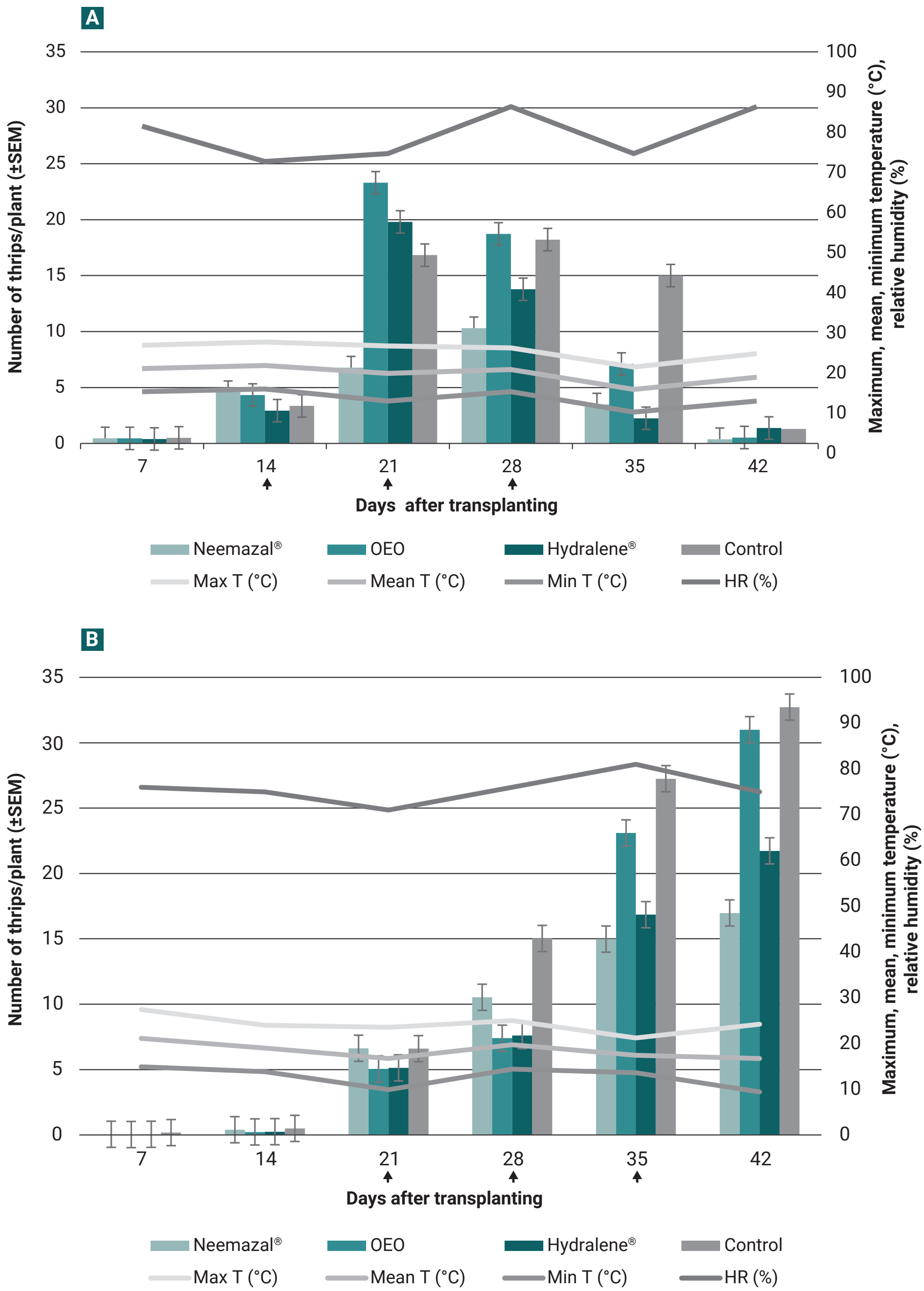

Figure 3. Mean number of thrips (± SEM) on plants in each treatment and meteorological data throughout the lettuce crop cycle in 2022 (a) and 2023 (b). Arrows indicate the date of biorational insecticides applications.

density of thrips caught on sticky traps showed significant differences among the traps positioned within the different treatments, in terms of the efficacy of bioinsecticides ($F_{3,204}$= 94,7, p≤ 0,01), and between the color of the traps ($F_{1,204}$= 2628,2, p≤ 0,01). Additionally, their interactions showed significant differences ($F_{3,204}$=3,67, p= 0,013) confirming the better attractivity of the blue color (fig. 5). The Neemazal® (103,1 ± 7,9) and OEO (93,5 ± 7,0) treatments had the highest number of thrips caught on blue traps, while the Hydralene® (75,6 ± 5,7) treatment had the lowest number of thrips caught compared to the control

Meteorological variables	2022		2023	
	April	May	April	May
Average maximum temperature (°C)	24,6	21,8	25,3	22,5
Average minimum temperature (°C)	12,4	9,6	13,2	11,4
Average mean temperature (°C)	18,5	15,7	19,3	17
Relative humidity (%)	77,4	82	75	77

Table 1. Meteorological data during the lettuce cycle in 2022 and 2023.

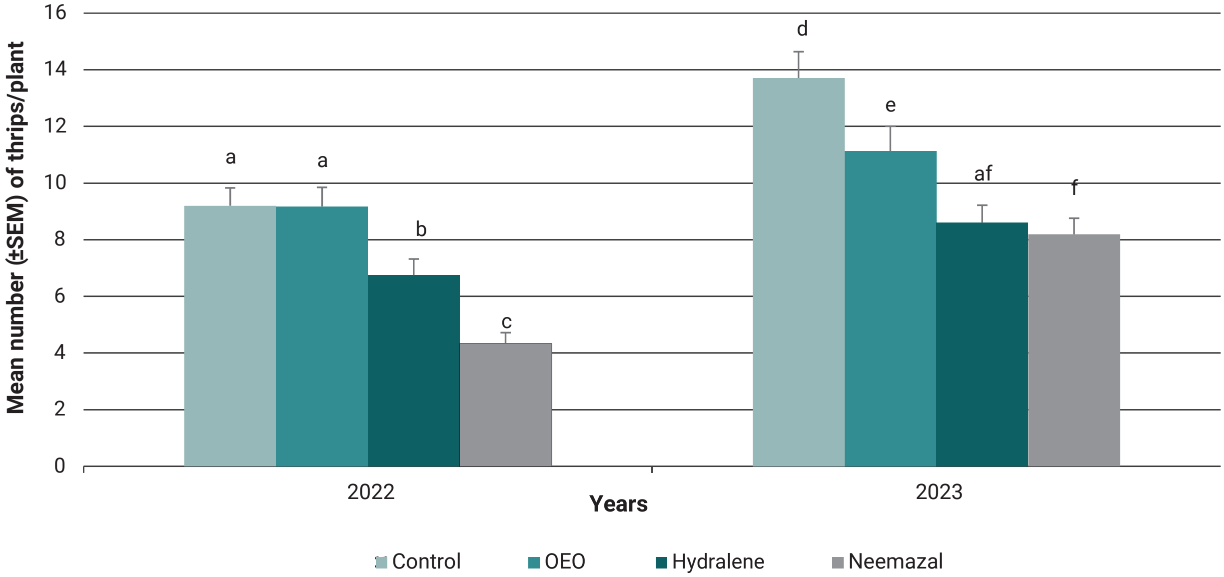

Figure 4. Mean number of thrips (± SEM) per lettuce plant considering interaction between effects of biorational insecticides and years (2022 and 2023) on the pest. Bars with different letters indicate significant differences using Tuckey test (α= 0,05).

(123,5 ± 9,1). All treatments caught a lower density of thrips on the yellow traps than the control. As occurred Hydralene® (27,1 ± 2,2) caught the lowest thrips density on yellow traps, but there were no significant differences between the OEO (37,3 ± 7,0) and Neemazal® (34,6 ± 2,8) treatments (fig. 5).

Regarding natural enemies, winged adults of *O. insidiosus*, coccinellids and syrphids were the predators recorded on blue and yellow sticky traps. The coccinellids population identified on the sticky traps was composed of native species such as *Coccinella ancoralis* (Germar), *Eriopis connexa* (Germar), *Colleomegilla quadrifasciata* var. *octodecimpustulata* (Mulsant) and *Hyperaspis festiva* (Mulsant). Syrphids identified on the sticky traps were represented by the species *Toxomerus* sp. and *Allograpta exotica* (Wiedemann) species. No significant effects on the abundance of *O. insidiosus* ($F_{3,204}$= 0,18, p= 0,91) and syrphids ($F_{3,204}$= 0,07, p= 0,97) were registered among treatments. Significant differences were recorded among treatments for coccinellids ($F_{3,214}$=2,9, p=0,03). OEO was the treatment that recorded the lowest mean catch of individuals on sticky traps (0,2 ± 0,1) compared to the control (0,4 ± 0,1), followed by Hydralene® (0,5 ± 0,1). No significant interactions were found between treatments and years ($F_{3,204}$=2,41, p=0,07).

Effects of treatments on lettuce yield

Logically, lettuce yields were higher in 2022 than 2023 with significant differences in gross (gl=1,F=34,4, p≤ 0,01) and marketable weight (gl=1,F=29,5, p≤ 0,01) between years. The Hydralene® treatment registered the lowest commercial weight in 2022 (130,7± 7,1 g/pl), while in 2023 it was recorded by the negative control (98,4 ± 4,9 g/pl). Conversely, the OEO treatment achieved the highest commercial weight in 2022 (167,2 ± 7,8 g/pl) and Neemazal® in 2023 (123,9 ± 5,5 g/pl). No significant differences were found between treatments, including the negative control (gl=3, F=2,5, p=0,09). The lowest percentage weight loss of lettuce was recorded in the Neemazal® treatment (5,2 ± 0,6% in 2022 and 5,6 ± 0,6% in 2023), intermediate in the Hydralene® (10,0 ± 0,8 in 2022 and 8,2 ± 0,9 in 2023) and

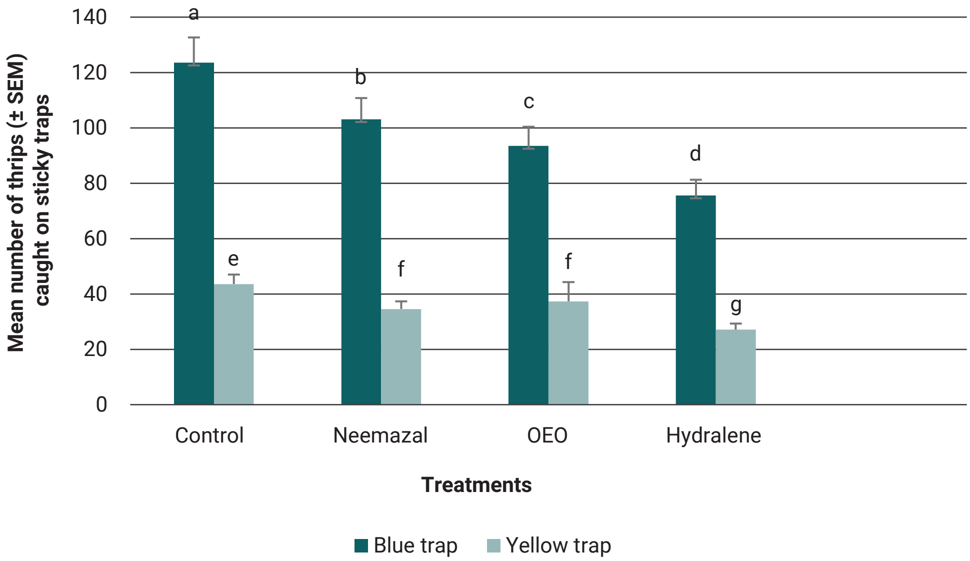

Figure 5. Mean number of thrips (± SEM) caught on blue and yellow sticky traps considering interaction between treatments and color traps (2022 and 2023). Bars with different letters indicate significant differences using Tuckey test (α= 0,05).

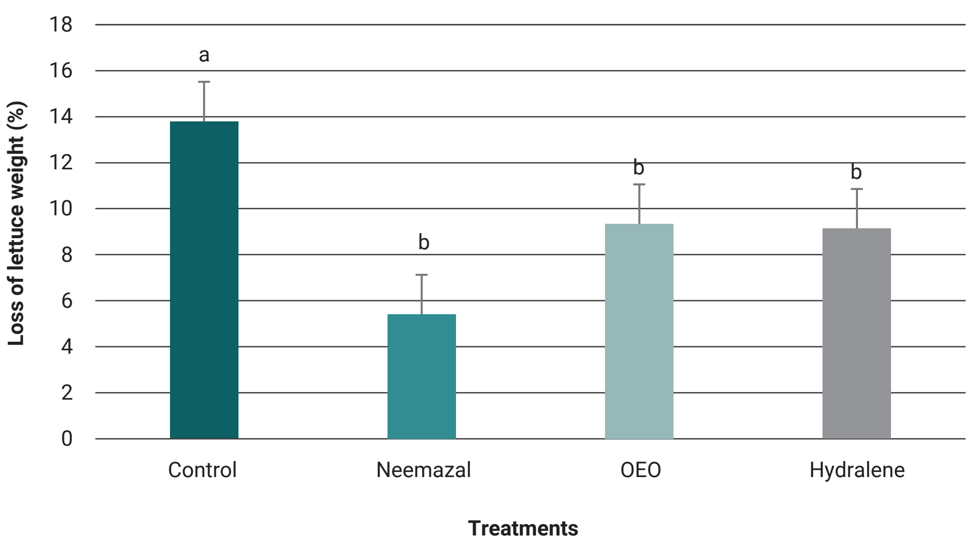

Figure 6. Overall mean (± SEM) loss of lettuce weight (%) under different biorational insecticides during 2022 and 2023. Bars with different letters indicate significant differences using Tuckey test (α= 0,05).

OEO (11,4 ± 1,1 in 2022 and 7,2± 0,9 in 2023) treatments and the highest in the negative control (15,9 ± 1,3 in 2022 and 11,7 ± 1,1 in 2023). The yield losses were found to be significantly lower in the biorational insecticide group when compared with the negative control (gl=3, F=11,09, p=0,0001) (fig. 6) and between years (gl=1, F=5,39, p≤0,03). However, no significant differences were found in the interaction between treatment and years (gl=3, F=1,16, p=0,34).

DISCUSSION

The present study was conducted with the aim of evaluating the efficacy of biorational insecticides against thrips and their effects on the yield of greenhouse-grown lettuce. It is important to note that in the study region, fall lettuce crops have a high pest pressure, but also a high market value, as their cultivation is limited in summer due to high temperatures. Consequently, farmers are faced with the challenge of carrying out efficient pest control while ensuring that the yield and commercial quality of the product meets acceptable standards and that it is safe for consumers and the environment. When it comes to the cost of insecticides, selective and biorational insecticides must compete with broad-spectrum insecticides because they are cheaper and guarantee farmers rapid pest control.

We have shown that the biorational insecticides tested in this study, except for OEO in 2022, were effective in reducing infestations of *Caliothrips phaseoli* and *Frankliniella* sp. in lettuce compared to the untreated control. However, no significant reduction in the mortality rate of the thrips population was observed in the short term (7 days) after each application. These results are consistent with those obtained by Palumbo (2022) who evaluated biopesticides efficacy against *F. occidentalis* on romaine lettuce under Arizona desert conditions. The longer time bioinsecticides take to drastically reduce pests compared to chemical active ingredients was previously highlighted by Shah *et al*. (2017) and this effect is more pronounced in lettuce due to its short crop cycle, as it occurred in this study (42 days from transplanting to harvest). Among the selective insecticides registered in Argentina, Spinosad (Tracer, Corteva, IRAC group 5) is derived from soil bacteria and is low in toxicity (toxicological class IV). It is highly effective for controlling thrips on lettuce. In an earlier series of trials conducted on lettuce in greenhouses at the Horticultural Section of INTA Concordia, applying Tracer® at a dose of 0,15 ml/l led to a significant reduction in the average thrips population, from 28 thrips per plant to three thrips per plant after a 72-hour application period (Diaz, unpublished data). These results contrast with those obtained in the treatments included in this study.

Significant differences in thrips population dynamics were observed between the 2022 and 2023 seasons. The effects of abiotic factors on thrips populations have previously been reported, with temperature, relative humidity and precipitation identified as the most significant influencing factors (Shannon *et al*., 2008; Da Silva *et al*., 2024). In this study, however, temperature and relative humidity did not have a significant effect on the thrips population. However, the optimum temperature for thrips development was estimated to be between 25-30°C, while the minimum temperature was found to be above 8°C (Reitz, 2009), which is consistent with the temperatures recorded during the 2022–2023 trials. Furthermore, the variations in temperature (developmental degree-days) and daily relative humidity seen from year to year might have affected the reproduction rate and, as a result, the population growth of the pest. It should be noted that, in the region where this study was conducted, insect nets covering greenhouses as a physical barrier are used only rarely, mainly due to the high temperatures throughout the year. Under these conditions, insect migration into greenhouses and dispersal are also influenced by biotic factors such as the availability and quality of cultivated and spontaneous vegetation around the greenhouses (Rodríguez and Coy-Barreras, 2023), as well as abiotic factors such as precipitation (Shannon *et al*., 2008). In this regard, the 2022 trial had almost twice as much precipitation and twice as many days with precipitation as the 2023 trial, which may have negatively impacted pest survival on external vegetation and limited their movement into the greenhouses.

Differences in thrips control on lettuce were registered among the treatments tested, showing that Neemazal® was the most effective bioinsecticide to reduce thrips infestation, resulting in a reduction in 42-53% of thrips/plant compared to the control. These results are consistent with those determined previously for *F. occidentalis*, highlighting the efficacy of Neemazal® compared to other biorational insecticides on different crops (Shah *et al*., 2017; Adom *et al*., 2024). In contrast, Hydralene®, which is made from potassium oleate, had a big impact on thrips infestations compared to the control. It was able to reduce the thrips population by 27-37%, which led to a decrease in yield of 8-10% because of the direct damage it caused. Thus, a recent study indicated that potassium salts of fatty acids showed a moderate toxicity against *Thrips parvisnipus* (Karny) on bean plants (*Phaseolus vulgaris* L. var. Roman) and was efficient to reducing feeding damage (Ataide *et al*., 2024). These results are consistent with those obtained in this study, in which Hydralene® showed an intermediate toxicity due to desiccation. A comparison was made of the toxicity of Neemazal® and Hydralene® at low thrips density/plant, as occurred in 2022. It was found that there were significant differences, while this effect was not observed in 2023 at higher thrips infestations (fig. 4). This result should be considered by growers when choosing bioinsecticide when considering the cost of the insecticide on the market. In this study, the cost of Hydralene® at the dosage used in this trial was 84,6 US$/ha, which is lower than the cost of Neemazal® (112,8 US$/ha). In addition, both biorational insecticides were expensive compared to Tracer® (44,7 US$/ha) at a recommended dose for lettuce (0,15 ml/l). In future studies, Tracer® should be evaluated with biorational insecticides as conventional positive control, as it is effective in thrips control.

This study showed that the (OEO)-based formulation had the lowest level of activity in controlling thrips (0-19%) on lettuce compared to the other tested formulations. A preliminary study showed that orange leaf oil had insecticidal activity against *Thrips flavus* (Schrank) under laboratory conditions (Pei *et al*., 2023), however, limited information is available for other thrips species under laboratory and field assays. In addition, the OEO tested in this study is an experimental formulation, and this study provides an initial evaluation of its efficacy against thrips on a field scale. There are few evaluations available for other soft-bodied insects, although further laboratory and field-scale studies are required.

A comparison of the three bioinsecticides tested in this study showed that Neemazal® and Hydralene® were best at controlling thrips in lettuce compared to OEO. These results are consistent with those registered for the control of thrips on pepper in a greenhouse (Sanad and Hassan, 2019). The differences in thrips toxicity between treatments tested in this study could be explained by the different and diverse modes of action of each active ingredient. For example, Neemazal® has multiple effects on insects including insecticidal, antifeedant and repellent activity (Schmutterer, 1990; Campos *et al*., 2016; Semere 2023). In this study, the reduction of thrips/plant could be related to the insecticidal and antifeedant effects of azadirachtin oil, reflected in low feeding damage on leaves and associated with the lower percentage of lettuce losses registered in this treatment (5%) compared to the control (11%).

In terms of yield, there were significant differences between treatments compared to the negative control, but not between treatments. However, looking at losses from an economic perspective, for a lettuce crop with a density of 12 plants/m^2, as used in this trial, the average yield losses over the two years of the trial were 95 kg/ha for Neemazal, 150 kg/ha for Hydralene, 174 kg/ha for OEO and 239 kg/ha for the control. Significant differences in the attraction of thrips to yellow and blue traps were found among the various treatments. It is known that thrips in greenhouses are strongly attracted to blue sticky traps with a reflectance wavelength of 450 nm, rather than yellow ones (Broughton and Harrison, 2012; Mi *et al*., 2019). In this study, thrips were captured on yellow traps at levels between 60% (Neemazal® and OEO) and 64% (Hydralene® and control) lower than on blue traps. Ren *et al*. (2020) found that visual cues dominate the behaviour of thrips at short distances. In this study, traps were placed 7 m apart between contiguous treatments within each bed and 2 m apart between beds. However, given that thrips have limited flight capacity, dispersing at a rate between 0,05 and 0,17 m/day (Rhainds and Shipp, 2004), there should be no interference with the recorded catches between treatments. On blue sticky traps, Neemazal® and OEO were the treatments in which the highest number of captures was recorded compared to control, being Hydralene® the treatment with the lowest mean density of thrips/trap. These results could be explained by the repellence effect caused by azadirachtin and the OEO, which could promote thrips leaving lettuce plants and then be attracted and captured on the sticky traps. Repellency is a highlighted attribute studied for several plant extracts against *F. occidentalis* (Ren and Chong, 2023). Previous studies observed a deterrent effect caused by azadirachtin on settlement of many insects on treated plants (Adusei and Azupio, 2022; Schmutterer, 1990). Similarly, Da Camara *et al*. (2015) concluded that the repellency exhibit by citrus oils was due to a synergistic action between the major (d-limonene) and the minor components of the essential oils and they registered this effect on *Tetranychus urticae* in laboratory and greenhouse experiments. From a management point of view, both colors of sticky traps help to reduce the overall population of thrips in greenhouses and are a practical tool for early detection of thrips infestations, which could improve the use of bioinsecticides (Rodríguez and Coy-Barrera, 2023).

In this study, a low occurrence of natural enemies on lettuce plants was detected in all treatments, including control plots. Therefore, a possible secondary effect of bioinsecticides on natural enemies cannot be confirmed, as reported in previous studies (Schmutterer, 1995 and Singh). However, on sticky traps adults of *O. insidiosus* were caught, two species of syrphids and five species of native ladybugs, showing no significant differences with the control treatment. The effect of OEO on the abundance of coccinellids caught in traps was high among the other compounds included in this study. Kimbari *et al*. (2010) indicate that a citrus sinensis essential oil caused variable mortality on another Coccinellidae, such as *C. septempunctata* and *Adalia bipunctata* L., but the effect of an essential oil on mortality or predatory ability can be species-specific (Giunti *et al*., 2022). Thus, Toledo *et al*. (2019) concluded that the essential oils increase the ability of *E. connexa* to prey *Myzus persicae,* but they had a neutral effect on *Coleomegilla maculata* (DeGeer). On the other hand, the least effect of biorational insecticides on coccinellids was registered with Hydralene® which acts by desiccation on soft-body insects, but on coccinellids, the elytra can protect adults from this active ingredient, as was described by Moss and Weed (2001). These results are expected to be confirmed in future laboratory trials using the insecticide formulation that was evaluated in this study.

Considering the results obtained in this study, we concluded that biorational insecticides are an effective tool to be integrated into an Integrated Pest Management (IPM) program for thrips. They provide safer active ingredients for the environment and beneficial fauna, while most importantly increasing the number of active ingredients that can be integrated into an action plan to reduce the risk of resistance. However, there are also some disadvantages to consider, such as a lower level of effectiveness, and lower persistence and the cost compared to conventional insecticides. Thus, a new generation of nanoformulations is currently being developed to increase their persistence at field scale and to improve their application (Iqbal *et al*., 2022; Laudani *et al*., 2022; Giunti *et al*., 2023). In addition, future research should focus on integration of biorational insecticides with established and innovative strategies, including soil practices, to achieve effective management of the thrips pest.

CONCLUSIONS

The efficacy of the biorational insecticides assayed in this study on thrips infesting lettuce showed that Neemazal® resulted in a significant reduction in thrips population on lettuce plants, followed by Hydralene®. The lowest efficacy was observed with the experimental formulation based on orange essential oil. The reduction in thrips infestation was directly related to the low percentage of crop losses due to minor damage to lettuce plants. In the experimental conditions of the study significant side-effects of the treatments on natural enemies were detected. Direct exposure of predators to biorational insecticides should be conducted in the future. At the first step of substitution of synthetic insecticides with biorational products Neemazal® and Hydralene® can be considered as promising candidates for inclusion in a rotation plan with conventional active ingredients to diminish resistance risks. In a more global approach, they could be integrated with the other control measures recommended in an IPM program, considering their advantages and disadvantages.

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