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ORIGINAL PAPER

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ORGANIC FOLIAR FERTILISERS CONTAINING CALCIUM, PHOSPHORUS, AND PLANT EXTRACTS FOR THE POTENTIAL CONTROL OF SOME INSECT PESTS OF *Brassica oleracea* var. *capitata* L.

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ABSTRACT

The cabbage whitefly (*Aleyrodes proletella* L.; Hemiptera: Aleyrodidae), the diamondback moth (*Plutella xylostella* L.; Lepidoptera: Noctuidae), and the cabbage aphid (*Brevicoryne brassicae* L.; Hemiptera: Aphididae) are responsible for the most significant losses in cabbage (*Brassica oleracea* var. *capitata* L.) cultivation. In this study, two commercial foliar fertilisers, Mitemine® (a calcium fertiliser) and D-Fense (a phosphorus and potassium fertiliser), and the insecticide Movento 100 SC (spirotetramat) were used to control these pests. In 2020, all treatments applied 6 times reduced pest infestations by approximately 60%–80% compared with the untreated controls. In 2021, a decrease in the number of treatments to 4 resulted in a 10%–20% reduction in effectiveness. Light and scanning electron microscopy revealed variations in the number and density of stomata, cuticle thickness, and leaf structure between the control and treatment groups. Anatomical evaluation suggested that the thickening of the cuticle and epidermis on the abaxial side of the leaves, including the vascular bundles – likely due to the calcium in Mitemine® – may be one of the mechanisms responsible for the observed decrease in the pest population density. The combination of D-Fense and Mitemine® did not significantly alter the effectiveness of Mitemine® against herbivores; however, this combination resulted in a more compact structure of the mesophyll, thicker abaxial epidermis inner cell walls, and a thick layer of cuticle on stomata surface. The findings indicate that foliar fertilisers containing calcium can enhance plant resistance to pests, offering a potential alternative to chemical pesticides in sustainable crop protection strategies.

Keywords: white cabbage, *Brevicoryne brassicae* L., *Aleyrodes proletella* L., *Plutella xylostella* L., mechanical barrier, sustainable crop protection

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is a globally significant food crop, with Poland ranking 13th in worldwide production [AtlasBig 2018–2025]. This cruciferous vegetable is an important part of many cuisines as well as a valuable source of nutrients, including vitamins C and K, fibre, and various antioxidants [Šamec et al. 2017]. However, Polish cabbage farms face significant challenges from climate changes, particularly rising temperatures, that promote fungal diseases and pest infestations, which

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can severely impact crop yields and quality [Hultgren et al. 2025]. The most economically important insect pests of white cabbage across the world are the cabbage whitefly (*Aleyrodes proletella* L., Hemiptera: Aleyrodidae) [Koca and Kütük 2020, Müller et all. 2024], the diamondback moth (*Plutella xylostella* L., Lepidoptera: Plutelllidae) [Wainwright et al. 2020] and the cabbage aphid (*Brevicoryne brassicae* L., Hemiptera: Aphididae) [Bisht and Kumar 2023]. These insect pests infest cabbages at different growth stages, causing a deterioration in crop quality and a reduction in yield. The cabbage whitefly can cause direct damage through feeding and indirect damage by promoting the growth of sooty mould on leaves [Łabanowski 2015, Hol and Kovaříková 2024]. The diamondback moth, particularly in its larval stage, can cause extensive foliar damage [Gautam et al. 2018], while the cabbage aphid forms dense colonies that can stunt plant growth and transmit viral diseases [Pal and Singh 2013]. Traditionally, these pests have been controlled primarily through the application of insecticides, but their widespread use has led to concerns about environmental impacts, human health risks, and the development of pesticide resistance in target species. Moreover, global trends and European Union directives – Directive (EU) 2023/959 of the European Parliament and of the Council of May 10, 2023 – are pushing for a significant reduction in chemical pesticide use, necessitating the exploration of alternative pest management strategies. In response to these challenges, researchers are investigating various approaches to reduce pest pressure while minimising chemical pesticide use [Shang et al. 2024]. One approach is to seek alternatives to traditional pesticides that work by restricting insect movement or respiration, or by causing death through drying and dehydration [Nile et al. 2019, Susurluk and Bütüner 2024]. Biological agents based on microorganisms, such as entomopathogenic fungi, entomopathogenic nematodes, the bacterium *Bacillus thuringiensis*, and viruses, are also used to control pest populations [Ramanujam 2019, Aynalem et al. 2022, Dede et al. 2022, Bütüner et al. 2024, Chaudhary et al. 2024, Yaraşır et al. 2024]. Another area of research focuses on substances that influence pest growth and development hormonally [Sarvar and Shad 2021]. The use of pheromones, repellents, and attractants is an important aspect of pest management strategies [Larsson 2016, Abd El-Ghany 2019].

There is also a growing focus on enhancing plant resistance to pathogens and pests through both structural and biochemical defences. This approach aims to make the plants themselves less susceptible to pest attacks, reducing the need for external interventions. Structural defences may include thicker cell walls and waxy cuticles that physically impede pest feeding or movement and improve the overall plant toughness and architecture [Seki 2016, Arora and Sandhu 2017, Marasek-Ciolakowska et al. 2021, Biswas et al. 2025]. Biochemical traits, such as odour and taste, may also contribute to insect non-preference [Sandhu et al. 2017]. Numerous studies have shown that the application of macro- and microelements can contribute to increase crop quality and yield, and to improve plant resistance or tolerance to insects [Bala et al. 2018, Tomić et al. 2020, Görlach and Mühling 2021]. Compounds that show promise in this regard include calcium, silicon, and phosphorus. Calcium strengthens plant tissue structures and plays a fundamental role in signalling and supporting plant defence responses [Thor 2019, Sarfraz et al. 2024, Singh et al. 2024, Wdowiak et al. 2024]. Similarly to calcium, silicon exerts physical effects on pests by accumulating in epidermal cells, reducing pest adaptability due to decreased digestibility and feeding preferences [Kvedaras et al. 2007, Massey and Hartley 2009, Strömberg et al. 2016, Frew et al. 2019]. Additionally, silicon plays a critical role in inducing plant biochemical defence mechanisms against pests [Gomes et al. 2005, Rahman et al. 2015]. Phosphorus is also vital because it enhances plant regeneration, root and shoot growth, collectively improving general resistance to insects [Bala et al. 2018].

The hypothesis for the present study is that mineral fertilisers strengthen cell walls, creating effective barriers against sap-sucking insects. This hypothesis is based on the understanding that nutrient management can significantly influence plant physiology and, consequently, pest interactions. Mineral fertilisers, which combine organic matter with essential minerals, may provide a balanced nutrient profile that supports both plant growth and defence mechanisms. To test this hypothesis, we assessed the effects of mineral fertilisers, applied alone or in combination, on pest population dynamics and changes in anatomical characteristics of leaves of *B. oleracea* var. *capitata* L. that may contribute to enhanced pest resistance. We applied two commercial foliar fertilisers – Mitemine® (a calcium fertiliser) and D-Fense (a phosphorus and potassium fertiliser) containing organic extracts of *Allium sativum* L., *Sesamum indicum* L., and *Salix alba* L. – to control the population of cabbage whitefly, cabbage aphid, and diamondback moth. Moreover, we discuss the possible increase in mechanical defences in white cabbage after foliar fertiliser application. By evaluating the efficacy of mineral fertilisers in pest management, this research contributes to the development of more sustainable agricultural practices. If successful, this approach could offer cabbage farmers an environmentally friendly tool to reduce pest damage while potentially improving crop quality and yield.

MATERIALS AND METHODS

Plant materials

White cabbage 'Ditmarska' plants were planted in sandy loam soil (soil quality class IVa, soil pH 5.7–6.0, and 1.7% organic matter) on 21 April 2020 and 17 May 2021, in the experimental field of the National Institute of Horticultural Research in Skierniewice, Poland (51°57'50.6"N, 20°10'15.2"E). The experiment employed a randomised block design with five replicates, with each plot comprising 40 plants (10 m^2 per plot). The plants were irrigated via a sprinkler system, as required during the experiments, with 20 mm cm^{-2} applied per irrigation event. Fertilisation consisted of phosphorus and potassium (superphosphate, 130 kg ha^{-1}; and potassium sulphate 160 kg ha^{-1}) applied pre-sowing. Nitrogen was applied pre-sowing (nitro-chalk, 130 kg ha^{-1}) and post-sowing (nitro-chalk, 70 kg ha^{-1}).

Experimental design

Four treatments were applied in 2020 and 2021 multiple times throughout the growing season, as detailed in Table 1. The control group (1) received no foliar fertiliser or pesticide treatment. The Mitemine® (Cosmocel SA) group (2) received this foliar calcium fertiliser (10% Ca and 14% CaO) along with plant extracts (2.5% *A. sativum* and 1.25% *S. indicum*). The group (3) received Mitemine® (1.5 L ha^{-1}) and D-Fense (Cosmocel S.A., 1 kg ha^{-1}) alongside the adjuvant Inex-A (50 ml 100 L^{-1} water). D-Fense is a phosphorus and potassium foliar fertiliser (31% P_2O_5 and 52% K_2O) that contains 4% of an organic extract from *S. alba* (at concentration of 1%) and 10,000 ppm phenolic acid. Inex-A is a non-ionic surfactant with wetting and penetrating action; it improves the distribution and spreading of substances on the surface and in the tissues of plants. The group (4) received Movento® 100 SC (Bayer) (100 g L^{-1} of spirotetramat, an insecticide) applied at 0.75 kg ha^{-1}.

Table 1. Description of the experimental treatments in 2020 and 2021

	Treatment	Dose	The number and dates of applications	
1	Control (no foliar fertilisers or pesticide treatment)	–	2020	2021
2	Mitemine®	1.5 L ha^{-1}	Six applications: 11, 18, and 25 May; 15, 22, and 29 June	Four applications: 12, 18, and 25 June; 2 July
	Inex-A	50 mL per 100 L of solution		
3	Mitemine®	1.5 L ha^{-1}	Six applications: 11, 18, and 25 May; 15, 22, and 29 June	Four applications: 12, 18, and 25 June; 2 July
	Inex-A	50 mL per 100 L of solution		
	D-Fense	1 kg ha^{-1}		
4	Movento 100 SC	0.75 L ha^{-1}	Two applications: 11 May; 15 June	Two applications: 12 and 25 June

Pest population assessment

Pest population assessments in both 2020 and 2021 were conducted on 25 randomly selected cabbage plants in each plot. The number of individuals of each species was recorded as they appeared on the same plants over time. The first observation was made when the plants were at the 9-leaf stage (BBCH 19), while the last observation was made when the heads reached 80% of the typical size (BBCH 48).

For the *A. proletella* the number of adults, egg batches, and larvae were counted. The counting of adult whiteflies was conducted early in the day, when temperatures were lower and the adults were less active. The first assessment was performed immediately before application, and the subsequent assessments were performed every 7 days, that is, on each application date. For *P. xylostella* the number of live caterpillars of different ages on all 25 plants was counted. The first assessment was performed immediately before application, while the subsequent assessments were performed every 7 days that is, on each application date. For the *B. brassicae* the number of living aphids on all 25 plants was counted. For both years, the first assessment was performed immediately before the application. In 2020, the second observation was made 3 days after the first application, whereas in 2021, it was made 7 days after the first treatment. The subsequent assessments were performed every 7 days, that is, each date of application.

The influence of the treatments on histological structure of leaves

Fourteen days after the last treatment, white cabbage plants were collected to assess the effects of foliar fertiliser on the anatomical traits of the leaves. To observe stomata with a light microscope (Eclipse 80i, Nikon, Tokyo, Japan), the abaxial epidermis was isolated from the third leaf using adhesive tape, 10 cm from their apices, and stained with 2% toluidine blue according to the method described by Dyki and Habdas [1996]. For each treatment, the stomatal density per square millimetre (n = 5 replications) and the stomatal length (n = 3 replications × 100 stomata) were determined. To observe the leaf anatomy, 10 mm × 5 mm pieces of the third leaf were cut for each treatment. The material was fixed with the CrAF (1% chromic acid, 1% acetic acid, and 50% formalin) solution for 48 h at room temperature, dehydrated using an ascending alcohol series (70%, 80%, 90%, and 100%), and embedded in paraffin according to a previously reported method [Marasek-Ciolakowska et al. 2020]. Transverse sections, 12 μm thick, were cut with a rotary microtome (Leica, Wetzlar, Germany) and stained with 1% safranin and 0.5% fast green. The sections were mounted in Canada balsam and analysed with the same light microscope that was used to observe stomata. For each leaf sample, the thickness of the lamina, the abaxial epidermal layer on the nerves and between the nerves, and the cuticle on the nerves and between the nerves was determined. For statistical analysis, seven replicates were used for each treatment, and each replicate consisted of 20 measurements. The surface and anatomy of leaf was examined using an Eclipse 80i microscope with the NIS-Elements BR ver. 2.30 program (Nikon, Tokyo, Japan) at 100× and 400× magnification.

Scanning electron microscopy was used to examine the leaf ultrastructure. Fragments of the third leaf (10 mm × 5 mm) were fixed with the CrAF solution, dehydrated in an ascending alcohol series (70%, 80%, 90%, and 100%), desiccated using critical point drying with CO_2, and sputter-coated with gold [Pathan et al. 2008]. There were three replicates for each treatment. The micromorphology of the leaf surface and the internal structure of the leaf was analysed using a JSM 6390LV scanning electron microscope (JEOL, Japan) at the Mossakowski Medical Research Centre, Polish Academy of Sciences in Warsaw.

Statistical methods

To analyse comprehensively the degree of plant infestation by pests throughout the experiment, the cumulative insect-day (CID) index was determined for each year [Ruppel 1983].

$$CID = \Sigma\ 0.5 \times (P_a + P_b) \times D_{a\text{-}b},$$

where P_a and P_b are the average number of insects per leaf on two successive sampling dates, and $D_{a\text{-}b}$ is the number of days between the two sampling dates.

The influence of various preparations on the histological structure of leaves and insect populations were analysed using one- or two-way analysis of variance (ANOVA; foliar fertiliser or pesticide treatment ×

study year). To stabilise variance, when necessary, the data were subjected to logarithmic transformation. The Newman–Keuls test was used to determine differences between the groups ($\alpha = 0.05$). Statistical analysis was performed with the STATISTICA v.13 program (StatSoft, Tulsa, OK, USA).

The efficacy of the applied treatments against pests was calculated using Abbott's formula:

$$\text{Efficacy (\%)} = \frac{\text{Infestation in control group} - \text{Infestation in treated group}}{\text{Infestation in control group}} \times 100.$$

RESULTS

The influence of the treatments on the pest populations

In both years of the study, the applied treatments reduced the population sizes of the studied insect species (Figs. 1–4) The efficacy of the treatments was similar in 2020 and 2021, with most exceeding 60% and, in some cases, reaching over 80% (Tab. 2).

In most cases, the two-factor ANOVA (treatment × year) did not reveal any statistically significant differences in the effectiveness of the tested protection programs in reducing the populations of the analyzed pest species. The only exception was observed for cabbage whitefly larvae, where the effectiveness of Movento 100 SC in 2021 was significantly higher compared to the combination of Mitemine® + Inex-A + D-Fens used in 2020. For the 'treatment' factor, the following F and p values were obtained: cabbage whitefly – adults: $F_{2,24} = 0.845$, $p = 0.442$; eggs: $F_{2,24} = 0.363$, $p = 0.700$; larvae: $F_{2,24} = 3.891$, $p = 0.034$; diamondback moth caterpillars: $F_{2,24} = 1.248$, $p = 0.305$; and cabbage aphids: $F_{2,24} = 0.333$, $p = 0.720$.

Aleyrodes proletella L.

In 2020, the first cabbage whitefly adults and eggs were recorded on cabbage on 15 June. The number of adults ranged from 1.9 to 2.7 individuals per plant (Fig. 1A), while the number of eggs ranged from 1.4 to 1.8 individuals per plant (Fig. 1C). Cabbage whitefly larvae were found both on the control plants and the plants treated with Mitemine® + Inex-A + D-Fense groups as late as 22 June, but their abundance did not exceed 1 individual per plant (Fig. 1E). In 2021, the first cabbage whitefly adults were observed on 12 June, and their abundance did not exceed 1 individual per plant (Fig. 1B). Besides the control plants, there was a small number of eggs found for the plants treated with Mitemine® + Inex-A + D-Fense (Fig. 1D). Larvae did not appear until 2 weeks later, that is, 24 June, at approximately 2 individuals per plant for the control plants and < 1 individual per plant for the treated plants (Fig. 1F).

Based on the two-way ANOVA (treatment × year), the treatment had a significant effect on the level of infestation by the cabbage whitefly, as measured with the CID. All the applied treatments similarly reduced the abundance of the cabbage whitefly compared with the control plants. This reduction occurred for the adults ($F_{3,28} = 32.948$, $p < 0.001$), eggs ($F_{3,28} = 18.585$, $p < 0.001$), and larvae ($F_{3,28} = 26.019$, $p < 0.001$) of this species. In addition, the pressure of the pest was significantly higher in 2021 than in 2020, ($F_{1,28} = 6.423$, $p = 0.017$ for adults, $F_{1,28} = 17.749$, $p < 0.001$ for eggs, and $F_{1,28} = 26.223$, $p < 0.0001$ for larvae) (Fig. 4A–C).

Plutella xylostella L.

In 2020, the first caterpillars of the diamondback moth were observed on 15 June, whereas in 2021, they appeared on 12 June. The initial infestation of the plants was higher in 2020, ranging from 2.3 to 4 individuals per plant, whereas in 2021, the number did not exceed 0.38 individuals per plant (Fig. 2). This difference was reflected later in the season, as the level of infestation, measured using the CID, was significantly higher in 2020 than in 2021 ($F_{1,28} = 111.654$, $p < 0.0001$). Over the 2 years of the research, there was a significant reduction in pest pressure, measured with the CID index, in the treated plants compared with the control plants ($F_{3,28} = 34.070$, $p < 0.0001$) (Fig. 4D).

Brevicoryne brassicae L.

In 2020, the first wingless cabbage aphids were observed as early as 11 May. Their abundance was low, ranging from 0.14 to 0.42 individuals per plant (Fig. 3A). Each treatment reduced the aphid population below the level observed in the control plants. However, 21 days after the third treatment, aphid abundance increased for all tested treatments, reaching an abundance ranging from 2.26 individuals per plant for

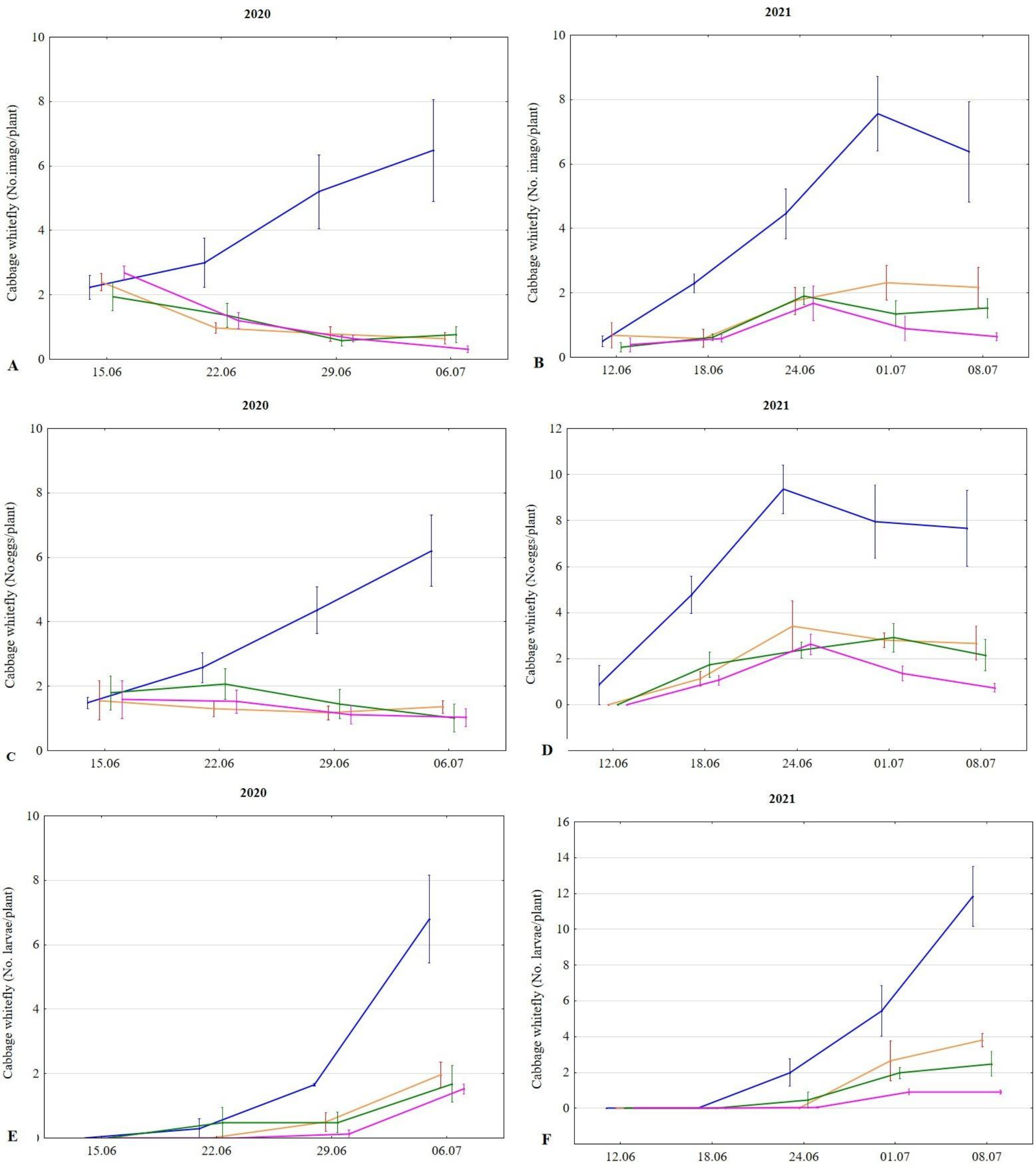

Fig. 1. The effect of the treatments on cabbage plant colonisation by *Aleyrodes proletella* L. (mean ± standard error of the mean) in (A, C, and E) 2020 and (B, D, and F) 2021. The panels show the number of (A and B) adults, (C and D) eggs, and (E and F) larvae. Legend: control; Mitemine® + Inex-A; Mitemine® + Inex-A + D-Fense; Movento100 SC

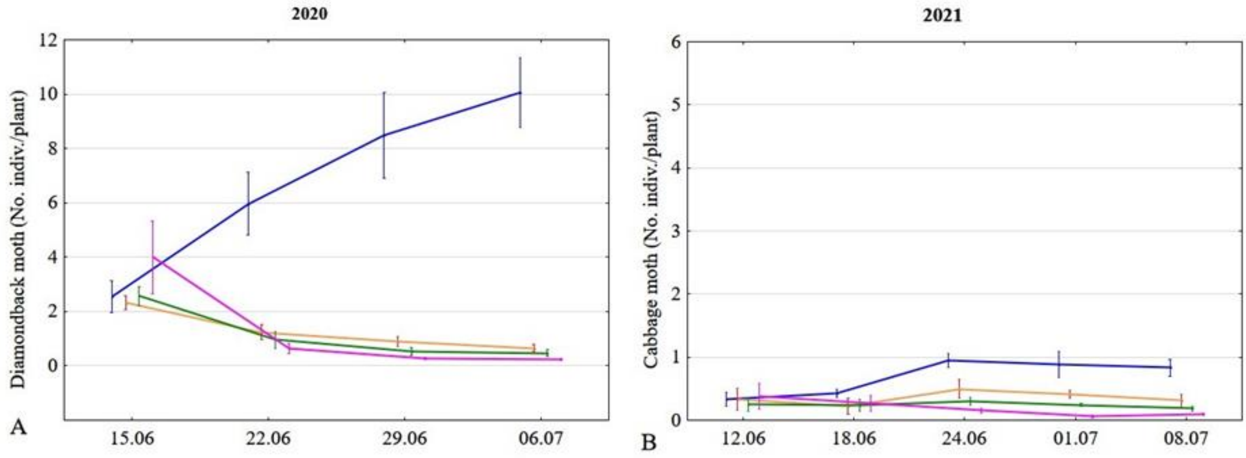

Fig. 2. The effect of the treatments on cabbage plant colonisation by *Plutella xylostella* L. (mean ± standard error of the mean) in (A) 2020 and (B) 2021. Legend: control; Mitemine® + Inex-A; Mitemine® + Inex-A + D-Fense; Movento100 SC

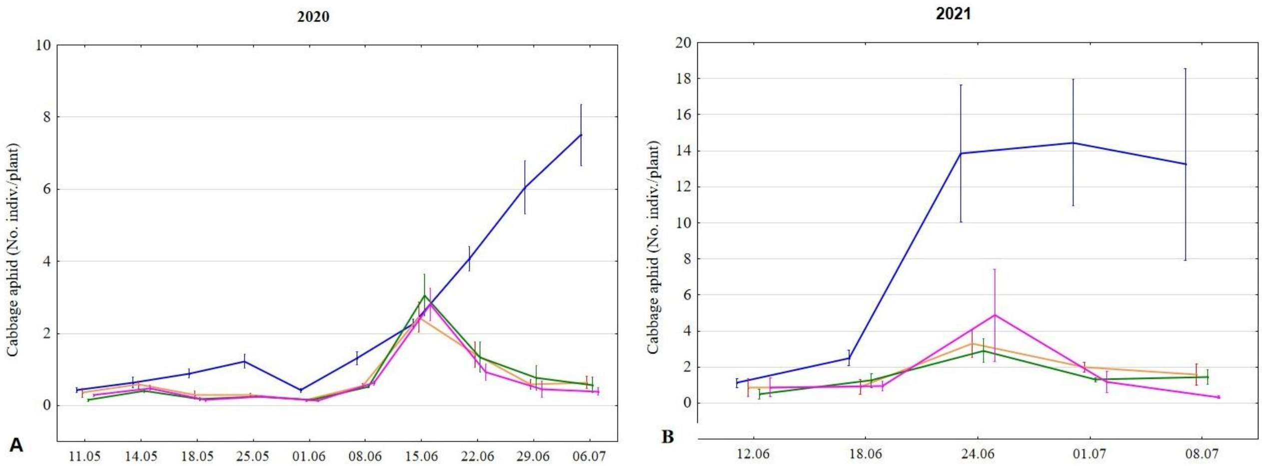

Fig. 3. The effect of the treatments on cabbage plant colonisation by *Brevicoryne brassicae* L. (mean ± standard error of the mean) in (A) 2020 and (B) 2021. Legend: control; Mitemine® + Inex-A; Mitemine® + Inex-A + D-Fense; Movento100 SC

the control plants to 3.06 individuals per plant for the plants treated with Mitemine® + Inex-A + D-Fense. A second spraying with Movento 100 EC, as well as a further application of fertiliser, reduced the abundance of cabbage aphids to < 1 individual per plant, while the abundance of aphids on the control plants increased to > 7 individuals per plant.

In 2021, cabbage aphids were found on plants on 12 June (Fig. 3B). The abundance ranged from 0.50 individuals per plant for the plants treated with

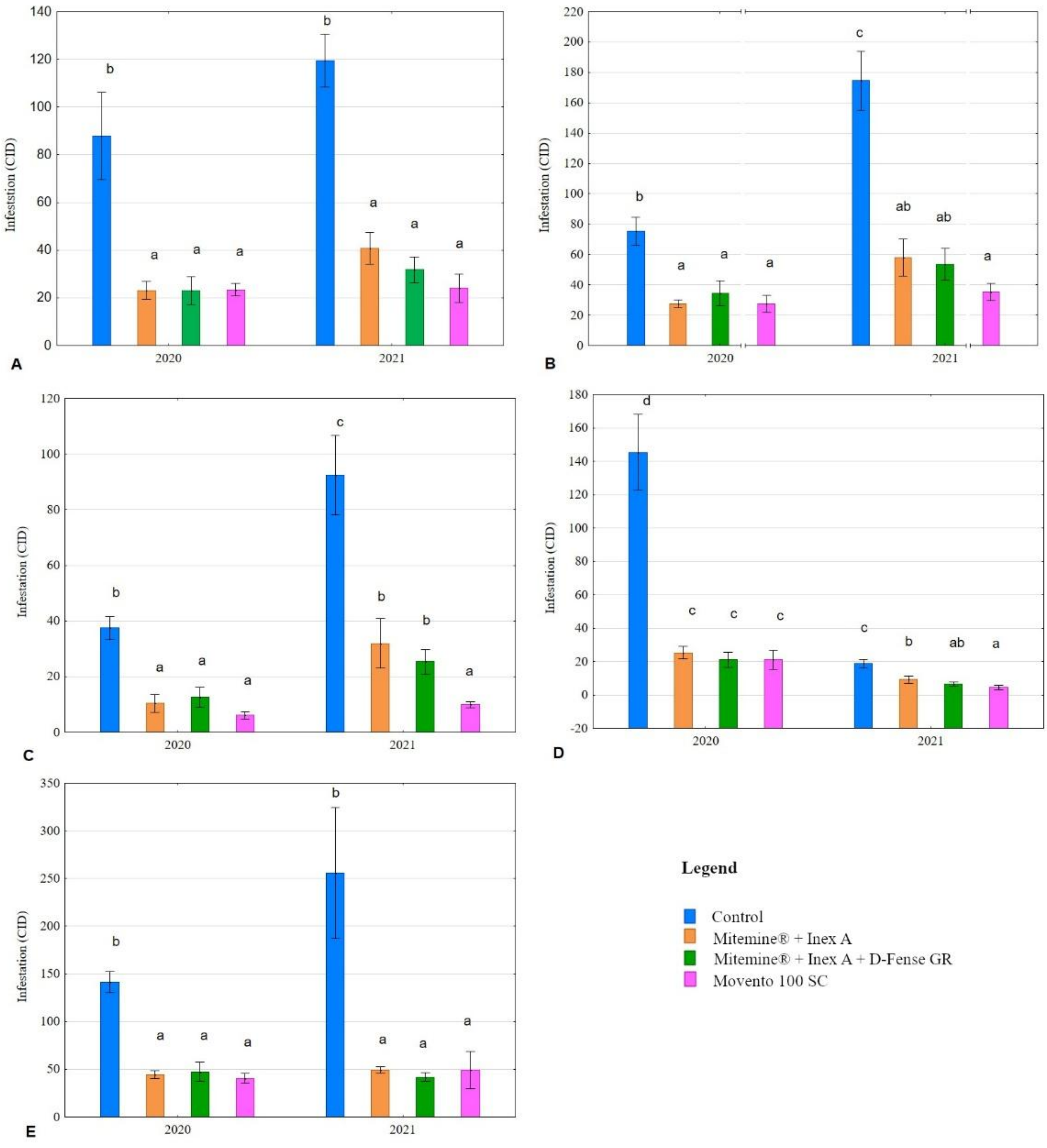

Fig. 4. The influence of the treatments on the mean (± standard error of the mean; n = 12) cumulative insect-days (CID) for cabbage whitefly (A) adults, (B) eggs, and (C) larvae; (D) diamondback moths; and (E) cabbage aphids in 2020 and 2021. For each graph, bars with the same letter do not differ significantly (two-way ANOVA followed by the Newman–Keuls test; $\alpha = 0.05$)

Table 2. Effectiveness of treatments against selected insect species in 2020 and 2021. Within each column, means followed by the same letter do not differ significantly (two-way ANOVA followed by the Newman–Keuls test; α = 0.05)

Treatment	Efficacy of treatments according to Abbott's formula (%)				
	Cabbage whitefly adults	Cabage whitefly egg batches	Cabbage whitefly larvae	Diamondback moth caterpillars	Cabbage aphids
2020					
Mitemine® + Inex-A	72.4 a	62.0 a	70.7 ab	80.4 a	68.1 a
Mitemine® + Inex-A + D-Fens	74.6 a	54.3 a	62.1 a	85.3 a	66.0 a
Movento 100 SC	70.2 a	58.7 a	83.8 ab	83.0 a	70.0 a
2021					
Mitemine® + Inex-A	65.7 a	63.0 a	72.4 ab	57.5 a	76.9 a
Mitemine® + Inex-A + D-Fens	73.9 a	69.0 a	70.7 ab	65.5 a	80.4 a
Movento 100 SC	78.6 a	77.5 a	89.0 b	75.6 a	82.5 a

Mitemine® + Inex-A + D-Fense to 1.12 individuals per plant for the control group. After 5 weeks, the abundance of aphids increased to 13.6 individuals per plant for the control plants, 1.58 individuals per plant for Mitemine® + Inex-A treatment, 1.43 individuals per plant for Mitemine® + Inex-A + D-Fense treatment, and 0.3 individuals per plant for Movento 100 SC treatment.

Two-way ANOVA revealed that the treatments significantly reduced the degree of cabbage aphid infestation measured with the CID index ($F_{3,28}$ = 26.554, $p < 0.0001$). Although the control plants showed higher infestation in 2021 than in 2020, the year effect was not significant ($F_{1,28}$ = 0.657, p = 0.425) (Fig. 4E).

The influence of the treatments on histological structure of the leaves

In the control plants, the difference between palisade and spongy mesophyll was clearly visible in the cross section (Fig. 5A). The cells of the spongy parenchyma were smaller than those of the palisade parenchyma and were loosely arranged. There treatments have variable effects on the anatomical structures of the leaves (Fig. 5B–H). In the leaves treated with Mitemine® + Inex-A or Movento 100 SC, the mesophyll cells had a looser structure and larger cellular spaces (Fig. 5B and D). In the cross-sections of the leaves treated with Mitemine® + Inex-A + D-Fense, the cells of the palisade and spongy mesophyll were tightly arranged, and small intercellular spaces were localised mostly above the stomata (Fig. 5C).

The treatments had a significant effect on the thickness of the cuticle of the lower side of the leaves ($F_{3,24}$ = 3.4188, p = 0.03342). This structure was the thickest for the leaves from the plants treated with Movento 100 SC and the thinnest for the leaves from the control plants. In addition, the leaves from the plants treated with Mitemine® + Inex-A or Mitemine® + Inex-A + D-Fense were thicker than the leaves from the control plants, although the differences were not significant (Fig. 6A).

For the cuticle on the vascular bundles of the lower side of the leaf, the only significant difference was between the leaves from the control plants and the leaves from the plants treated with Mitemine® + Inex-A ($F_{3,24}$ = 3.9594, p = 0.01997) (Fig. 6B). A significant difference in the thickness of the cuticle of the lower side of the leaves ($F_{3,24}$ = 3.9594, p = 0.01997) has been found. Specifically, in the leaves from the control plants, this structure was significantly thinner

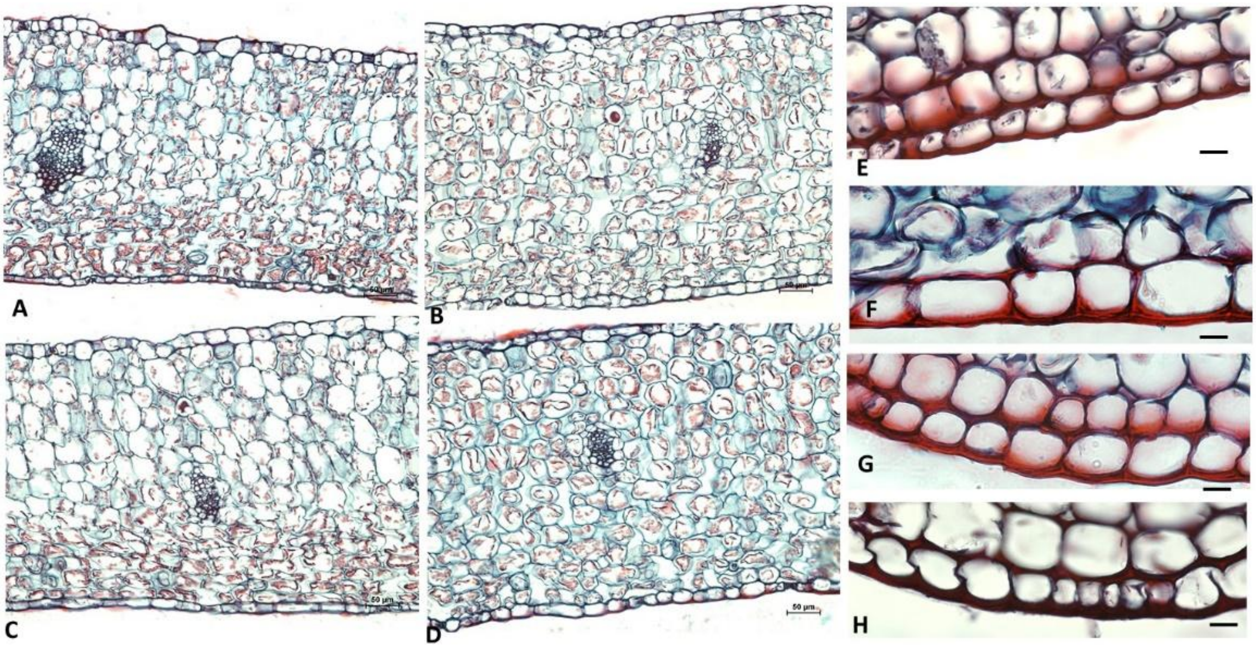

Fig. 5. Anatomical traits of white cabbage leaves stained with safranin-fast green and observed under a light microscope. (A–D) Cross-sections of a leaf blade. (E–H) The size of abaxial epidermis cells at the vascular bundles. The scale bars represent 10 µm. (A and E) Untreated (control); (B and F) Mitemine® + Inex-A; (C and G) Mitemine® + Inex-A + D-Fense; (D and H) Movento 100 SC

compared with the leaves from the plants treated with Mitemine® + Inex-A or Movento 100 SC (Fig. 6C). The tretments had a significant effect on the cuticle of the abaxial side of the leaves measured on the vascular bundles ($F_{3,24}$ = 8.0860, p = 0.00068): the epidermis was thicker for the vascular bundles of the leaves from the plants treated with all tested preparations compared with the leaves from the control plants (Fig. 5E–H and 6D). The veins of the leaves from the plants treated with Mitemine® + Inex-A showed the thickest skin, but it was not significantly different compared with the leaves from the plants treated with Mitemine® + Inex-A + D-Fense or Movento 100 SC.

The treatment also significantly affected the stomatal density ($F_{3,16}$ = 58.595, p = 0.00000) and length ($F_{3,16}$ = 38.694, p = 0.00000). The leaves from the control plants had a significantly higher stomatal density compared with the leaves from the plants that were treated with the tested preparations (Fig. 6E). In contrast, the leaves from the plants treated with Mitemine® + Inex-A + D-Fense or Movento 100 SC presented longer stomata (Fig. 6F).

The anatomy of the lower (abaxial) leaf surface of white cabbage was examined with a light microscope and a scanning electron microscope (Fig. 7). Staining of the isolated abaxial epidermis with toluidine blue (for examination with a light microscope) revealed the presence of a thick cuticle layer on some stomata in the epidermis of the leaves from the plants treated with Mitemine® + Inex-A + D-Fense (Fig. 7C). The scanning electron micrographs of the leaf showed that leaf surface was not flat, but rather consisted of bumps/protrusions (Fig. 7E–H). There were anisocytic, elliptical, and outlined stomata below the epidermal surface (sunken stomata). Similarly to the observations made under the light microscope, there were differences in the surface ultrastructure for the leaves from the plants treated with Mitemine® + Inex-A + D-Fens, where the thick layer of cuticle-covered stomata and protrusions were less distinct (a less folded epidermis) (Fig. 7C). There was no difference in the leaf surface ultrastructure between the control plants and the plants treated with Mitemine® + Inex-A or Movento 100 SC, except for the stomatal size and density.

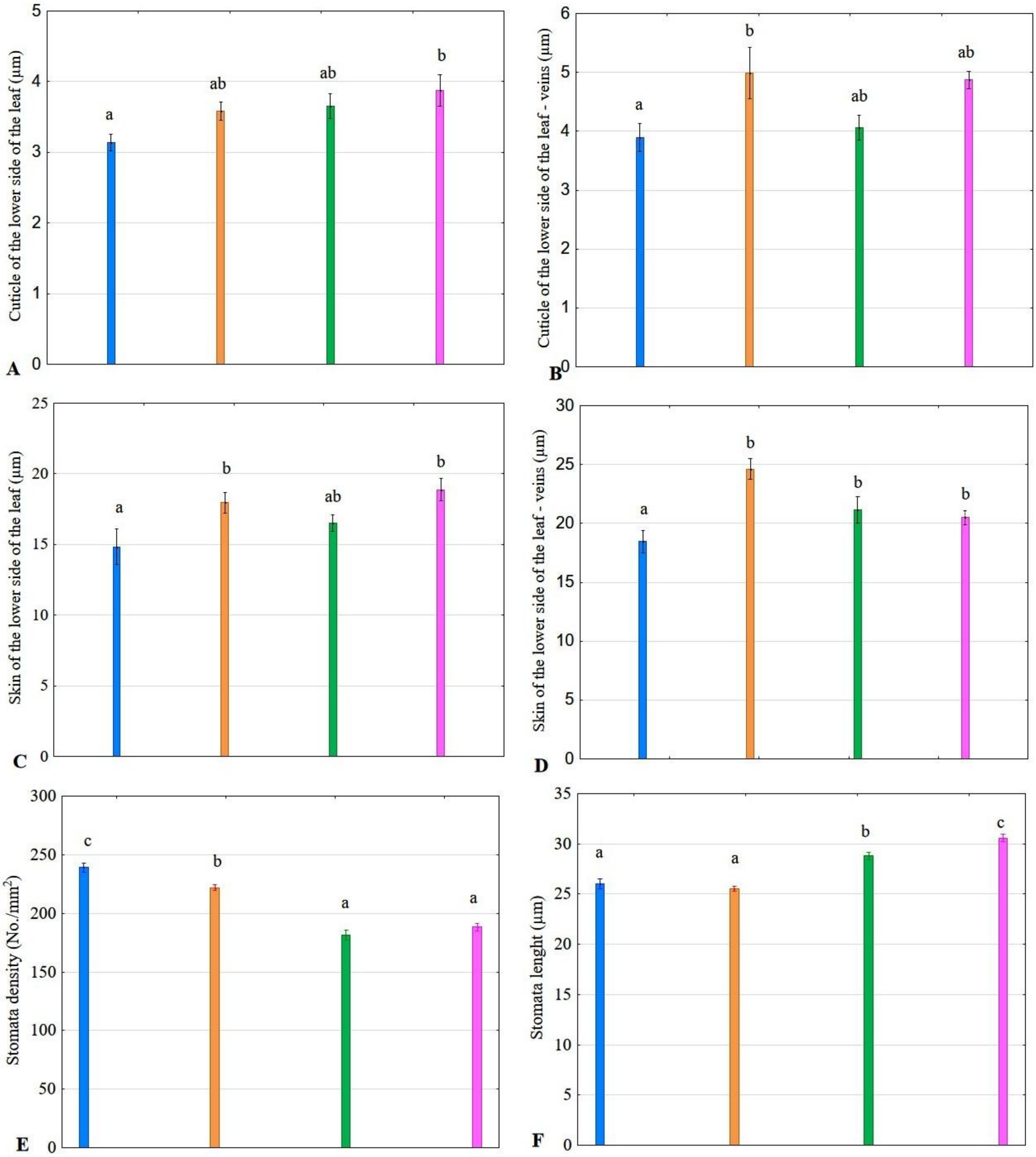

Fig. 6. The influence of the treatments on the histological structures of the lower side of white cabbage leaves. The graphs show (A and B) the cuticle, (C and D) the skin, and (E and F) the stomata. For each graph, the bars with the same letter do not differ significantly (one-way ANOVA followed by the Newman–Keuls test; α = 0.05). Legend: Control; Mitemine® + Inex-A; Mitemine® + Inex-A + D-Fens; Movento 100 SC

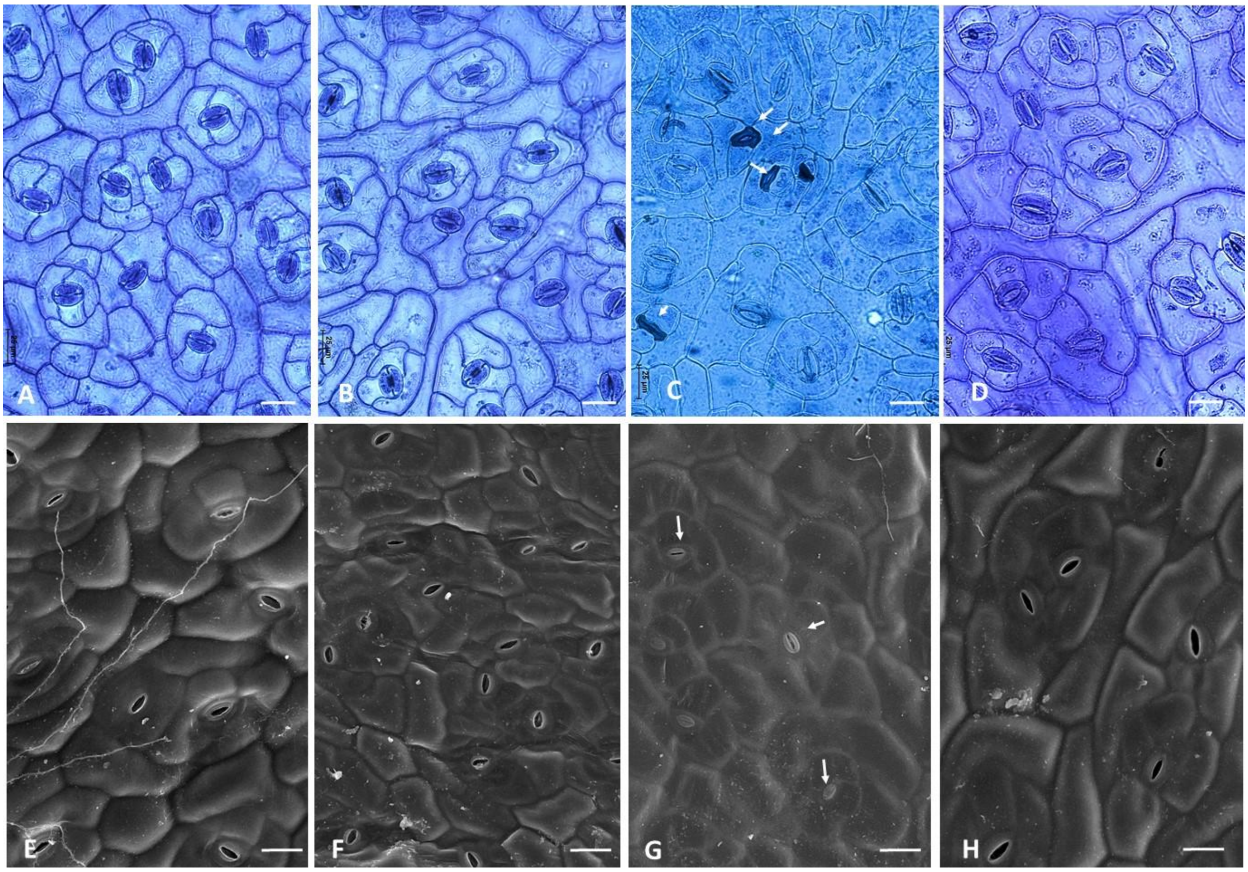

Fig. 7. The influence of the treatments on the abaxial epidermis of white cabbages as observed with (A–D) a light microscope and (E–H) a scanning electron microscope. The images are from (A and E) control plants, (B and F) plants treated with Mitemine® + Inex-A, (C and G) plants treated with Mitemine® + Inex-A + D-Fense, and (D and H) plants treated with Movento 100 SC. The white arrows indicate stomata with thick cuticle layers. The scale bars represent 25 μm

In the leaf cross-sections observed under a scanning electron microscope (Fig. 8), the leaves from the plants treated with Mitemine® + Inex-A + D-Fense presented more turgid and rigid cell walls compared with the leaves from the plants treated with the other preparations. In the leaves from the plants treated with Mitemine® + Inex-A or Movento® 100 SC, the mesophyll cells had a looser structure and larger cellular spaces.

One-way ANOVA revealed significant differences in the epidermal wall thickness between the treatments ($F_{3,36}$ = 59.108, p < 0.00001). The leaves from the plants treated with Mitemine® + Inex-A + D-Fense had thicker inner cell walls of the lower epidermis (0.91–1.68 μm) compared with the leaves from the control plants (0.34–0.40 μm) and the plants treated with the other preparations (0.54–1.05 μm) (Fig. 8G). The palisade and spongy mesophyll cells of the leaves from the plants treated with Mitemine® + Inex-A + D-Fense were tightly arranged (Fig. 8C).

DISCUSSION

We have demonstrated that the foliar fertiliser Mitemine®, applied alone or in combination with D-Fense as a spray six or four times during the growing season, could effectively control cabbage whiteflies, diamondback moth caterpillars, and cabbage aphids. Its effectiveness was comparable to the ref-

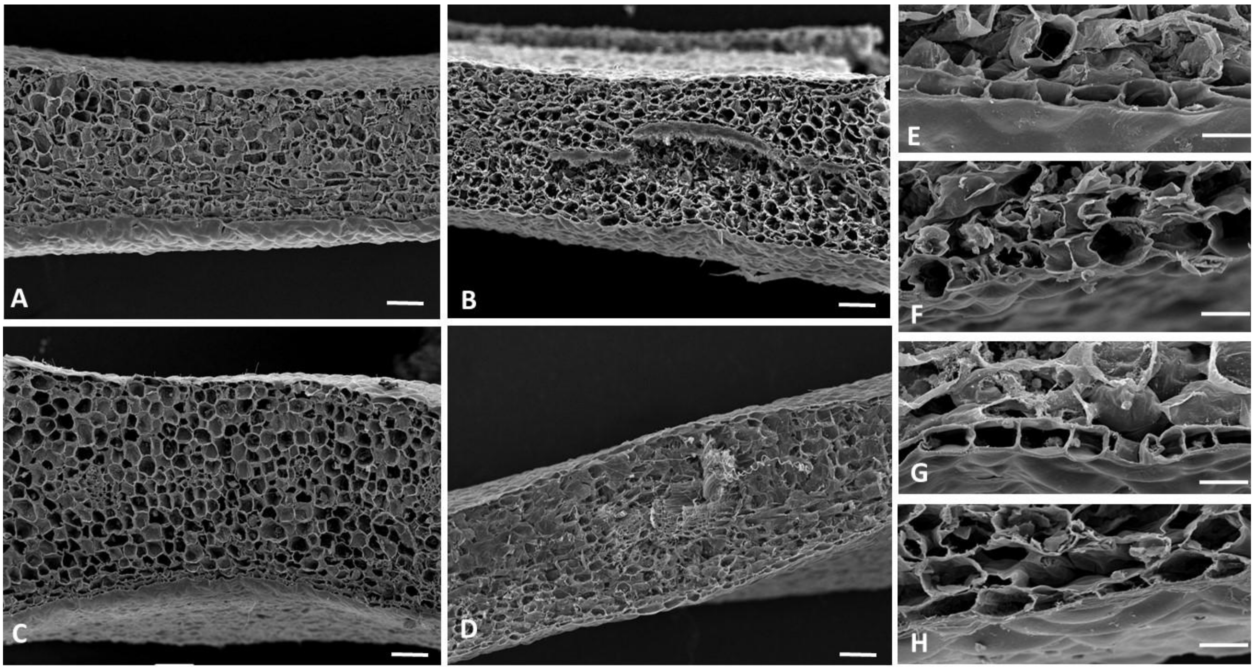

Fig. 8. Scanning electron micrographs of white cabbage leaves: (A–D) cross-sections through the leaf and (E–H) the abaxial epidermis at a higher magnification. The images are from (A and E) control plants, (B and F) plants treated with Mitemine® + Inex-A, (C and G) plants treated with Mitemine® + Inex-A + D-Fense, and (D and H) plants treated with Movento 100 SC. The scale bars represent 100 µm for panels A–D, and 20 µm for panels E–H

erence product Movento 100 SC (containing spirotetramat), which was applied twice during the growing season.

The effect of Mitemine® on insects may be due to the presence of calcium compounds in its formulation and extracts from *A. sativum* (garlic) and *S. indicum* (sesame). The insecticidal and repellent properties of these plant extracts have been demonstrated in several insect species. The insecticidal effect of sesame leaf extract has been reported against *Callosobruchus maculatus* (Fab.) [Negbenebor et al. 2022] and *Clavigralla tomentosicollis* Stål [Negbenebor et al. 2020], whereas garlic essential oil is effective against the adult cereal moth *Sitotroga cerealella* (Olivier) [Yang et al. 2012]. In turn, an ethyl acetate extract from garlic repels the beetles *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motsch.) [Ho and Ma 1995]. These bioactive extracts may act synergistically with calcium compounds to enhance the overall efficacy of Mitemine®, thus providing a comprehensive approach for pest control. The role of calcium as a secondary messenger in multiple signalling pathways to promote both innate immunity and adaptive stress responses in plants has been demonstrated by many researchers [Thor 2019, Parmagnani and Maffei 2022]. Because the phloem is essential for transporting nutrients throughout the plant, it is protected from pests in various ways. Plants defend their phloem primarily through physical barriers and chemical signals. Physical defences prevent insects from reaching nutrients. According to Seki [2016], the resistance of *Dianthus caryophyllus* (L.) to *Tetranychus urticae* (Koch) is related to the thickness of the palisade tissue. Similarly, a tight cell arrangement of mesophyll tissue in savoy cabbage and kale cultivars has been linked to low infestation by *A. proletella* [Marasek-Ciolakowska et al. 2021]. As a result of damage to plant tissues by phloem-feeding insects, there is an increase in calcium ion (Ca^{2+}) levels in plant cells. This leads to the formation of callose, which blocks the phloem tubes and stops the flow of nutrients [Fu et al. 2022]. Moreover, during stress, the Ca^{2+} concentration in plant cells increases, activating multiple signalling pathways, including those related to jasmonic acid (JA), as well as the activation of genes responsible for lignin synthesis, thereby strengthening cell walls [Denness et al. 2011, Vélez-Bermúdez et al. 2015]. The increased thickness of plant structures we observed in the present study, including the increase in the epidermal and cuticle thickness of vascular veins, appears to be consistent with these mechanisms.

In contrast, insects feeding on phloem sap try to break this line of defence by releasing enzymes and proteins through their saliva. These proteins either degrade defence proteins or bind to Ca^{2+} to inhibit callose synthesis [Will et al. 2013]. Consequently, this can lead to calcium deficiencies in insect foraging areas. It is possible that the use of calcium compounds in our experiment effectively increased the available calcium levels, thereby facilitating the induction of plant resistance to the tested pests.

Modification of the leaf structure, characterised by a decrease in the density and elongation of the stomata occurred after the Mitemine® treatment, may have created an additional barrier to the insects. We have previously confirmed the important role of leaf morphological and anatomical features in the resistance of savoy cabbage (*B. oleracea* var. *sabauda*) and kale (*B. oleracea* var. *sabellica*). Specifically, we found that the cuticle structure, folding of epidermal cells, and stomatal size and density are significant determinants of susceptibility to cabbage whiteflies [Marasek-Ciolakowska et al. 2021].

We also investigated whether adding D-Fense could enhance the effectiveness of Mitemine® in reducing the population of the tested pests. D-Fense contains phosphorus, potassium, an organic *S. alba* extract, and phenolic acids. Phosphorus and potassium, which are essential macronutrients, play a significant role in plant growth. Phosphorus is crucial for energy metabolism, nucleic acid production, cell signalling, and strengthening the cell wall. Potassium regulates water balance by controlling the movement of stomata, stabilising enzymes, and alleviating the effects of stress [Amtmann et al. 2008, Marschner 2012, Sardans and Peñuelas 2021]. Other components of D-Fense are organic white willow extract and phenolic acids, which are thought to have significant biological potential [Deniau et al. 2019]. *Salix alba* extract contains salicin, a precursor of salicylic acid, which plays a significant role in inducing systemic acquired resistance in plants against pathogens. Feng et al. [2021] showed that when applied exogenously, salicylic acid can enhance plant defences against pests with a piercing-sucking mouth apparatus. Under controlled conditions, Ramniwas et al. [2024] showed that an *S. alba* extract reduces feeding and egg laying by oriental fruit fly *Bactrocera dorsalis (Hendel)*, inhibits larval development, and increases the mortality of this insect. The conversion of salicin to salicylic acid may be the key mechanism that triggers resistance in both pathogens and pests. This dual mode of action makes *S. alba* extract a promising alternative to chemical pesticides, especially in the context of sustainable agricultural systems.

Despite the potentially beneficial properties of D-Fens, we found that adding D-Fense to a mixture of Mitemine® and Inex-A did not significantly enhance the effectiveness of pest reduction (Fig. 4A–E). Based on these results, we conclude that the key factor in increasing the resistance of cabbage plants to pests is the combination of Mitemine® and Inex-A.

We found that foliar application of Movento 100 SC, a preparation containing the active substance spirotetramat, had a significant effect on the histological structure of white cabbage leaves, including thickening of the cuticle and epidermis, as well as a reduction in the stomatal density, and an increase in the stomatal length. Spirotetramat belongs to Insecticide Resistance Action Committee (IRAC) group 23. It is distributed systemically in plants and disrupts metabolic processes related to lipid synthesis, which affects the development and reproduction of insects [Brück et al. 2009]. The effect of spirotetramat on the structural characteristics of leaves is surprising, but some studies have indicated that this compound leads to notable physiological and biochemical changes in plants. For example, in cucumbers, spirotetramat increases the activity of antioxidant enzymes, including superoxide dismutase, catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase, and phenylalanine ammonia-lyase [Homayoonzadeh et al. 2022]. Additional biochemical analyses revealed an increase in the content of some amino acids, sucrose, glucose, and fructose. The concentrations of salicylic acid and minerals, such as calcium, manganese, copper, zinc, iron, nitrogen, and magnesium, are elevated in spirotetramat-treated plants. It is worth noting that salicylic acid can affect the structure of plant tissues, including the structure of the epidermis, the thickness of the cuticle, and the development of the vascular system, because it acts as a phytohormone involved in defence reactions and regulation of plant growth. In tomatoes, Mandal et al. [2009] analysed the effect of exogenous salicylic acid on resistance to pathogens, and found that it strengthens the plant cell wall by increasing lignification. However, the stomatal density may be affected by the nutrient status. Researchers have noted a reduction in the stomatal frequency in lemons [Eichert and Fernández 2023], and a decrease in the stomatal pore sizes and apertures in peaches and pears, as a consequence of iron deficiency [Fernández et al. 2008]. It is possible that the reduction in the stomatal density and the increase in the stomatal length, we observed after applying spirotetramat to the white cabbage plants, may result from the complex physiological and biochemical mechanisms induced by this compound.

Although spirotetramat was the most effective against *B. brassicae*, *A. proletella* larvae, and *P. xylostella* caterpillars, its use is limited to conventional cabbage crops only. Thus, there is a need to search for new products to reduce the occurrence of those pests in both conventional and organic crops. Undoubtedly, the present research using the fertiliser Mitemine® to reduce pests should be classified as an appropriate step in this direction. Regardless of the mechanisms behind the pest population reduction, the inclusion of fertiliser-based products as an alternative to spirotetramat represents a significant step towards sustainable crop protection by reducing environmental impacts and promoting long-term soil and ecosystem health. Although fertilisers can improve plant health and resilience, unlike spirotetramat, they do not provide immediate pest control. Moreover, their use requires more applications, which results in increased direct costs and environmental burdens. Despite these challenges, the use of foliar fertilisers for plant protection against pests remains a valuable approach worth incorporating into modern conservation practices.

CONCLUSIONS

Our 2-years of research using a multi-pest approach has provided robust evidence for the potential of the tested treatments in integrated pest management strategies for cabbage cultivation. Mitemine®, when applied six times with the addition of an adjuvant (Inex-A), reduced the infestation of cabbage plants by *B. brassicae*, *A. proletella*, and *P. xylostella* with effectiveness that was comparable to that of the Movento 100 SC (containing the insecticide spirotetramat), which was applied twice. The addition of D-Fense did not significantly alter the effectiveness of Mitemine®. The results indicate that one of the mechanisms responsible for reducing the pest population density after applying Mitemine® may be thickening of the cuticle and epidermis on the underside of the leaves, including the vascular bundles, which is likely an effect of the calcium present in Mitemine®. We also found that spirotetramat may induce changes in leaf structure similar to those observed after the application of Mitemine®. The observed anatomical modifications offer intriguing insights into the possible mechanisms of induced pest resistance, opening up new avenues for research in plant–pest interactions, and the development of innovative crop-protection strategies. As agricultural systems face increasing challenges from pest pressure and climate change, such multifaceted approaches to crop protection that enhance plant resilience through structural and physiological modifications may play a crucial role in ensuring food security and sustainable agricultural practices.

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CONFLICTS OF INTEREST

The funders (Osadkowski Company) had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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