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INFLUENCE OF SEVERAL METHODS OF FLOWER AND FRUITLET THINNING ON THE YIELD AND QUALITY OF GALA MUST APPLES

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ABSTRACT

Apple trees of the Gala Must, grafted onto the dwarfing M.9 rootstock, planted in 2014 at a spacing of 3.5×1.8 m, were subjected to flower and fruitlet thinning in 2022–2024. Seven thinning combinations were used: 1 – (M) Mechanical thinning of flowers at the stage when the petals had emerged in 2 or 3 flowers in the inflorescence, using the German BAUM device; 2 – (C) Chemical thinning of fruitlets with Globaryll 100 SL containing cytokinin; 3 – (H) Hand thinning of fruitlets after June drop; 4 – (M+C) Mechanical thinning of flowers with the BAUM device supplemented by chemical thinning of fruitlets as in pt. 2; 5 – (M+H) Mechanical thinning of flowers with the BAUM device supplemented by hand thinning of fruitlets after June drop; 6 – (C+H) Chemical thinning of fruitlets with Globaryll 100 SL supplemented by hand thinning after June drop; 7 – (Control) Trees in which neither flowers nor fruitlets were thinned out.

In most treatments, the thinning of flowers or fruitlets caused a significant decrease in fruit yield but improved fruit quality, compared with the control. The thinning treatments increased the weight and size of apples, as well as their soluble solids content. Most apples were of a favourable marketable size in the range of 7.0–7.5 cm. The combined thinning treatments (M+H, M+C, C+H) resulted in the production of too many overgrown apples, which are known to be more susceptible to bitter pit, which in turn may reduce their storage life. Most of the thinning treatments resulted in a higher soluble solids content in the fruit without a significant effect on their firmness.

Keywords: apple tree, BAUM device, mechanical thinning, yielding, fruit quality

INTRODUCTION

The thinning of flowers or fruitlets is commonly used in fruit growing. In the case of apple trees, regulating fruiting is a very important treatment in commercial orchards. Many apple cultivars tend to produce an excessive number of flower buds, very abundant flowering and fruit setting. The fruitlets compete with one another for the limited range of assimilates supplied by the tree. Removing unnecessary flowers or fruitlets improves the leaf-to-fruit ratio. This allows a better supply of assimilates to the remaining fruitlets on the tree. In order to obtain good quality apples, 20–30 leaves per fruit are necessary [Seehuber et al. 2014]. If fruiting is too abundant, the apples produced are small and of low commercial value [Solomakhin and Blanke 2010].

When apple trees blossom very abundantly, it is enough for about 7% of the flowers to set fruit to obtain a profitable marketable yield of good quality [Untiedt and Blanke 2001]. Mechanical or chemical thinning of flowers or fruitlets is therefore necessary to obtain high-quality fruit [Blanke 2008], to reduce the labour consumption of subsequent hand thinning of fruitlets

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and, consequently, to prevent trees from switching into biennial bearing [McArtney et al. 1996].

Fruit growers need to remove excess amounts of flowers or fruitlets from apple trees annually [Peifer et al. 2018]. Looney [1993] stated that activities aimed at obtaining the optimal number of fruitlets on the tree are the most important agrotechnical treatments in apple cultivation, influencing the high quality of apples (mainly increasing their size, improving the economic efficiency of the orchard, and ensuring appropriate flowering in the following year, which ensures regular fruiting) [Greene and Costa 2013, Solomakhin et al. 2012]. In addition, thinning out flowers helps maintain a balance between vegetative growth and fruiting in apple trees [Dennis 2000].

In general, the aim of thinning is to produce fewer fruits [Link and Blanke 1998], but of better quality. Thinning flowers or fruitlets is time-consuming and expensive, therefore experiments are conducted to improve it. Thinning can be performed at various phenological stages of tree development, from flowering to when the fruitlets are as large as 18 mm in diameter [Greene and Costa 2013].

In practice, three main methods are used for thinning fruitlets or flowers of fruit trees: by hand [Embree et al. 2007, Hampson and Bedford 2011], by chemical means [Basak 2000, Dennis 2000, Wertheim 2000, Dorigoni and Lezzer 2007, McArtney and Obermiller 2010], and by mechanical means [Bertschinger et al. 1998, Schupp et al. 2008, Solomakhin and Blanke 2010, Seehuber et al. 2014, Lordan et al. 2018].

Hand thinning of apple fruitlets is most often done after the period of the natural drop of fruitlets, which usually occurs about 6 weeks after the trees have reached the full-bloom stage [McArtney et al. 1996]. This method of fruit thinning is the most reliable, most accurate and most effective, but also the most expensive and very time-consuming [Menzies 1980, Costa et al. 2013], and performing it after the natural drop of fruitlets may negatively affect fruit size in a given year and flowering in the following year [Dennis 2000, Fallahi and Greene 2010].

During hand thinning of fruitlets, the smallest, russeted, pest-damaged and deformed fruits are removed, and 'fruit clusters' are thinned out. Even if hand thinning of fruitlets does not result in the required improvement in fruit size, it does have an impact on the more abundant setting of flower buds for the following year.

Thinning of fruitlets by hand is now performed less and less frequently due to the high labour consumption and high costs [Schupp et al. 2008, Martin-Gorriz et al. 2012]. It is becoming increasingly difficult to carry out hand thinning from year to year due to decreasing labour availability [Strijker 2005, Greene and Costa 2013]. Hand thinning of fruitlets is therefore a supplementary treatment that follows chemical thinning, which is not always fully sufficient [Menzies 1980].

In apple and pear cultivation, chemical thinning of fruitlets is commonly used to improve fruit quality and prevent biennial bearing [Tromp 2000, Whiting and Ophardt 2005]. When chemically thinning flowers or fruitlets, the final effect depends largely on the weather conditions during the treatment [Robinson and Lakso 2011, Costa et al. 2013, Lordan et al. 2018], the age of the trees, the intensity of flowering, and the apple cultivar [Wertheim 2000, Greene and Costa, 2013], as well as the active substance of the preparation used, its dose and the date of the treatment.

Fruit growers are reluctant to use chemicals for thinning during or just after flowering because of the risk of late spring frosts, which can significantly reduce the yielding of trees. The optimal solution seems to be the use of preparations containing the growth regulator benzyladenine (BA) for the late thinning of apple fruitlets. This treatment is performed when apple fruitlets are 10–12 mm in diameter, or even 15–18 mm in the case of some cultivars. This stage usually occurs 2–4 weeks after flowering [Basak et al. 2013]. At that time, the percentage of fruit-setting can already be reliably estimated.

In order to obtain positive results when thinning fruitlets with preparations containing benzyladenine, warm and humid weather is necessary during the treatment and preferably for the next few days. The minimum temperature should be around 18°C, and preferably 20–25°C. For the effectiveness of the treatment, the optimal temperature is more important than the stage of fruitlet development [Buban 2000].

When planning a thinning strategy, on the one hand it would be good to perform the treatment relatively early – then it has the greatest impact on the formation of flower buds for the following year [Wertheim 2000], but on the other hand, with late thinning, when

it is already obvious how many fruitlets will remain on the tree, it is easier to decide which chemicals and in what doses should be used for thinning.

Great concern for food safety together with environmental awareness have limited the availability of chemical thinning agents. Due to the effectiveness of chemical thinning, which depends mainly on weather conditions, as well as the impossibility of using it in organic farming, and in the case of hand thinning also due to the lack of workers and high labour costs, attempts are being made to introduce treatments using various types of devices for mechanical thinning of flowers [Damerow et al. 2007, Solomakhin and Blanke 2010, Basak et al. 2013, Kon et al. 2013, Seehuber et al. 2014, McClure and Cline 2015, Theron et al. 2016].

The mechanical thinning of flowers is a more environmentally friendly technology, an alternative to traditional, standard chemical and hand thinning of fruitlets and is another method that improves the regularity of fruiting. The effectiveness of mechanical flower thinning, unlike chemical thinning, is less dependent on weather conditions, the cultivar, or the age of trees [Dorigoni et al. 2010]; it also requires less time and is cheaper than thinning by hand. Mechanical thinning allows complete elimination or very significant reduction in the doses of the chemical preparations used for thinning, which is very beneficial when introducing eco-friendly or integrated production methods.

Most studies have shown that mechanical thinning of flowers reduces their numbers on the tree and improves the quality of fruit at harvest [Solomakhin et al. 2012, Lordan et al. 2018]. The way tree crowns are trained, and also the growth vigour of a given apple cultivar, is crucial to achieving the expected results when using devices for mechanical flower thinning [Bertschinger et al. 1998, Schupp et al. 2008, Pflanz et al. 2016]. In most commercial orchards, trees are nowadays trained in the form of a spindle-shaped (conical) leader crown. In this form, the lateral shoots extending from the leader in the lower part are the longest, and those at the top the shortest. Densely planted orchard trees trained in the form of a slender spindle crown are suitable for mechanical flower thinning [Schupp et al. 2008, Hampson and Bedford 2011].

During mechanical thinning, flowers are knocked to the ground or damaged together with young leaves. This stimulates the release of ethylene in the shoots, which also additionally promotes the subsequent drop of fruitlets after the treatment. Mechanical flower thinning is most effective when performed from the time of the full opening of 2–3 flowers in the inflorescence, but it can also be performed over a longer time, from the pink bud stage until the end of flowering [Veal et al. 2011, Hehnen et al. 2012, Solomakhin et al. 2012, Kon et al. 2013].

The effects of mechanical flower thinning are visible soon after the treatment, so they can be corrected if necessary after the trees have flowered by spraying them, for example, with agents containing benzyladenine, or by thinning the fruitlets manually [Schupp et al. 2008, Basak et al. 2013, Kon et al. 2013]. Hehnen et al. [2012] showed that combining mechanical thinning of flowers with hand or chemical thinning of fruitlets helped to obtain optimal results in terms of flower thinning and fruit quality in some apple cultivars.

The mechanical thinning of flowers with the BAUM device manufactured in Germany, which was designed for trees trained in the form of a spindle crown, allows a significant reduction in the labour costs needed to perform hand thinning of fruitlets. Using this method allows the grower to become largely independent from the traditional thinning methods, i.e. chemical and by hand. It is a simple, cheap, and effective procedure. Mechanical thinning, although not as popular as chemical or hand thinning, is certainly a real alternative to the other methods used.

Fruit growers are reluctant to conduct early thinning of flowers with mechanical devices due to the risk of late spring frosts, which can significantly reduce the yielding of trees [Hampson and Bedford 2011], and also due to the increased risk of fire blight on such trees, caused by the possible penetration of the pathogen into the plant through damaged bark after the mechanical thinning of flowers [Ngugi and Schupp 2009]. In addition, mechanical flower thinning can damage young leaves on the spurs (less intense photosynthesis), which play an important role at the beginning of fruit growth [Bertschinger et al. 1998, Ngugi and Schupp 2009, Solomakhin and Blanke 2010, Greene and Costa 2013, Basak et al. 2013, McClure and Cline 2015, Win et al. 2023]. Flowers that have been injured during mechanical thinning and have not fallen from the trees may develop misshapen, uneven fruit, but this is rare.

The aim of this study was to develop a mechanical method of flower thinning and to compare this approach to chemical and manual thinning of fruitlets.

MATERIAL AND METHODS

The experiment was conducted in 2022–2024 and assessed the effectiveness of mechanical thinning of apple blossoms in comparison with hand thinning of fruitlets and chemical thinning of fruitlets with a preparation containing cytokinin. The study on the thinning of flowers and fruitlets of apple trees was conducted in the Experimental Orchard of the Institute of Horticulture – State Research Institute in Dąbrowice, on apple trees of the cultivar Gala Must, grafted onto the M.9 rootstock and planted in 2014 at a spacing of 3.8×1.5 m. The trees were trained in the form of a conical spindle crown. The experiment was established on a podsolic soil, with a mechanical composition defined as slightly loamy sand, soil quality class IVb.

In 2022–2024, spring temperatures were exceptionally moderate, with many sunny days and not much rainfall. The trees blossomed and bore fruit quite abundantly every year. The earliest flowering of the trees was recorded in 2024, around the 10th of April. At the time of spraying the trees with Globaryll 100 SL, intended for thinning fruitlets, the air temperature was 20°C, and in the following days above 20°C, and there was no wind. Average air temperatures and precipitation totals from April to September in 2022–2024 are shown in Table 1. Globaryll 100 SL is a plant growth regulators containing the natural hormone benzyladenine (BA), which belongs to the cytokinin group.

During the growing season, the experimental plot was subjected to the necessary orchard maintenance work consisting of standard treatments: fertilization, irrigation, weeding, and spraying the trees against diseases and pests.

The experiment was set up in a block design, with four replications. Each experimental plot consisted of five consecutive trees in a row.

Mean fruit weight was calculated from the weight of a fruit sample divided by the number of apples in that sample.

Fruit size measurements were performed by calibrating according to the diameter, with division into size grades every 0.5 cm. The size grades ranged from 6.0 cm to 8.5 cm.

Measurements of fruit colour (red blush coverage) were based on a 1–4 scale: 1 – fruits with blush covering up to 25% of the surface, 2 – blush covering 25% to 50% of the surface, 3 – blush covering 50% to 75% of the surface, 4 – fruits with blush covering more than 75% of the surface.

Mean fruit weight, size and colour development were determined on samples of 4 standard 20 kg crates of apples from the evaluated combinations.

Table 1. Average air temperature and total precipitation from April to September in 2022–2024

Parameter	April	May	June	July	August	September
			2022			
Average air temperature (°C)	5.3	13.2	18.3	18.3	19.9	11.0
Total precipitation (mm)	24.4	36.8	68.4	116.6	82.0	39.4
			2023			
Average air temperature (°C)	7.6	11.4	17.1	19.4	20.2	16.3
Total precipitation (mm)	49.4	41.6	30.2	61.6	95.0	12.8
			2024			
Average air temperature (°C)	10.5	16.3	18.7	20.6	19.4	16.2
Total precipitation (mm)	23.8	39.8	48.6	23.2	30.6	33.2

Measurements of fruit firmness and refraction were taken immediately after harvest on 10 representative apples from each replication, using a hand-held Effegi firmness meter (Fruit Pressure Tester, FT 327, T.R. Turoni Srl, Italy). The measurements were taken twice on each fruit, on the blush side and on the opposite side.

Refraction (soluble solids content) was determined on the same fruit used to measure fruit firmness. The measurements were performed using an electronic refractometer (Pocket Refractometer PAL-1, ATAGO, Japan).

For the mechanical thinning of flowers, a BAUM-type device was used, developed in Germany in 2007 [Damerow et al. 2007], which was adapted to trees trained in the form of a spindle crown. The BAUM device has the ability to remove flowers located in the crown close to the tree leader, and not only in its peripheral zones [Veal et al. 2011]. Removing flowers from the depths of the crown is a desirable procedure because those flowers produce fruits of lower quality [Kong et al. 2009]. The BAUM flower thinner is a small device, easy to transport, working with a tractor equipped with a hydraulic lift. It is equipped with an arm from which 3 horizontally positioned rotors extend, which can be set in any position (changing both the height and the angle of inclination). During thinning, they enter between the tree branches. The rotors rotate around their own axis and have thin plastic cords installed on them that knock down the flowers [Basak et al. 2013].

The following combinations of flower and fruit thinning were used annually:

1. Mechanical thinning of flowers at the end of April, at the stage of open flower petals in two or three flowers in the inflorescence. The procedure was performed with the BAUM device, at a tractor working speed of 5 km·h^{-1} and a rotation speed of the flower knocking rotors of 300 rpm (M).

2. Chemical thinning of fruitlets in the last ten days of May, when fruitlets had reached a size of about 10–12 mm. The treatment was performed with Globaryll 100 SL containing cytokinin, at a dose of 1.5 l·ha^{-1} (C).

3. Hand thinning of fruitlets in mid-June, after the June drop. One fruitlet was left in the cluster, at intervals of approx. 10–15 cm (H).

4. Mechanical thinning of flowers with the BAUM device supplemented by chemical thinning of fruitlets with Globaryll 100 SL on the dates as above (M+C).

5. Mechanical thinning of flowers with the BAUM device supplemented by hand thinning of fruitlets after the June drop (M+H).

6. Chemical thinning of fruitlets with Globaryll 100 SL supplemented by hand thinning of fruitlets after the June drop (C+H).

7. The control consisted of trees in which neither flowers nor fruitlets were thinned out (Control).

The obtained results were statistically processed using the variance analysis method. Duncan's test was used to assess the significance of differences between means at a significance level of 5%.

RESULTS AND DISCUSSION

In most treatments performed in our study, the thinning of flowers or fruitlets caused a significant decrease in fruit yield and improvement in fruit quality in comparison with the control. In some combinations, there were no significant differences in fruit yield and quality between the mechanical flower thinning and chemical fruitlet thinning when compared with the control trees. The thinning treatments of flowers and fruitlets of the Gala Must apple trees reduced the yield of apples, depending on the year and combination, in relation to the control trees within the range of 7.2 to 57.0% and caused an increase in mean apple weight from 3.8% to as much as 63.5% (Tables 2–4).

The greatest reduction in the percentage of fruit set (by approx. 48.0%) in 2023–2024 was caused by mechanical flower thinning supplemented by hand thinning of fruitlets, and the smallest (7.0 to 17.0%) in 2022–2024 in the combinations where only chemical or mechanical thinning was performed. Similar results of mechanical flower thinning had been obtained by Solomakhin and Blanke [2010], Basak et al. [2013], Schupp and Kon [2014], McClure and Cline [2015], and Lordan et al. [2018].The cumulative fruit yield for the three-year study period was significantly lower for the trees in the M+H and C+H combinations (Table 4).

Each method of flower and/or fruitlet thinning, except the chemical thinning of fruitlets alone, caused a significant increase in mean fruit weight (Tables 2–4). The lowest increase in mean fruit weight in the Gala Must was recorded in the combinations where only the chemical thinning of fruitlets was performed (3.8 to 4.9%) and also where only mechanical flower thinning was applied (22.0 to 32.3%).

Table 2. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the yield and mean weight of apples in 2022

Treatments	Yield (kg·tree^{-1})	Yield (t·ha^{-1})	Yield reduction (%)	Mean weight of apple (g)	Increase in mean weight of apple (%)
Control	36.7 ±0.58 b*	64.4	–	123 ±1.73 a	–
M	30.3 ±0.88 b	53.1	17.5	150 ±4.62 b	22.0
M+H	19.2 ±1.73 a	33.7	47.7	174 ±2.60 cd	41.5
M+C	15.8 ±0.33 a	27.7	57.0	161 ±0.33 bc	30.9
C	34.0 ±2.60 b	59.6	7.5	129 ±4.91 a	4.9
C+H	16.2 ±1.15 a	28.4	55.9	178 ±2.31 d	44.7
H	20.7 ±0.88 a	36.3	43.6	157 ±3.76 b	27.6

* M – mechanical thinning of flowers, C – chemical thinning of fruitlets, H – hand thinning of fruitlets.
Means in columns followed by the same letter are not significantly different at the $p = 0.05$ level of significance.

Table 3. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the yield and mean weight of apples in 2023

Treatments	Yield (kg·tree^{-1})	Yield (t·ha^{-1})	Yield reduction (%)	Mean weight of apple (g)	Increase in mean weight of apple (%)
Control	51.4 ±0.88 c*	90.2	–	96 ±2.60 a	–
M	47.7 ±2.89 c	83.7	7.2	127 ±3.76 b	32.3
M+H	27.0 ±2.03 a	47.4	47.5	149 ±4.91 c	55.2
M+C	35.8 ±0.88 b	62.8	30.4	133 ±3.76 b	38.5
C	45.1 ±2.31 c	79.1	12.3	100 ±0.00 a	4.2
C+H	33.0 ±2.03 ab	57.9	35.8	157 ±3.46 c	63.5
H	34.0 ±0.88 ab	59.6	33.9	157 ±2.60 c	63.5

* For explanations, see Table 2.

Table 4. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the yield and mean weight of apples in 2024, and the cumulative fruit yield for 2022–2024

Treatments	Yield (kg·tree^{-1})	Yield (t·ha^{-1})	Total yield for years 2022–2024 (kg·tree^{-1})	Yield reduction (%)	Mean weight of apple (g)	Increase in mean weight of apple (%)
Control	44.5 ±1.15 c*	78.1	132.6 ±12.71 c	–	106 ±1.15 a	–
M	38.8 ±2.02 b	68.1	116.8 ±15.30 bc	12.8	132 ±2.60 b	24.5
M+H	22.8 ±0.58 a	40.0	69.0 ±6.94 a	48.8	156 ±2.89 c	47.2
M+C	26.0 ±0.88 a	45.6	77.6 ±17.32 ab	41.6	137 ±4.91 b	29.2
C	40.4 ±2.31 bc	70.9	119.5 ±9.87 bc	9.2	110 ±1.73 a	3.8
C+H	25.1 ±0.58 a	44.0	74.3 ±14.45 a	43.7	163 ±4.33 c	53.8
H	26.8 ±0.88 a	47.0	81.5 ±11.27 ab	39.8	160 ±1.45 c	50.9

* For explanations, see Table 2.

Kon et al. [2013] noted a decrease in apple yield by over 50% and an increase in mean fruit weight by 28 g as a result of mechanical flower thinning, compared with fruit from control trees. Kong et al. [2009], Veal et al. [2011], and McClure and Cline [2015] demonstrated in their experiments that mechanical flower thinning with the BAUM device of several apple cultivars limited excessive yielding of trees, improved fruit quality, and prevented biennial bearing.

In 2023 and 2024, in the control combination and where the fruitlets on the trees were thinned out only chemically, there was as much as 34.2 to 60.2% of small fruits (up to 6.5 cm in diameter), depending on the combination. In all the other combinations of flower and/or fruitlet thinning, no more than 12.6% of apples were in this size range (Tables 6–7). In the same years, in the control combination and where only chemical thinning of fruitlets was performed, the percentage of fruits with a diameter above 6.5 cm ranged from 39.8 to 65.8%, whereas in all the other combinations, after the thinning of flowers and fruitlets, at least 87.4% of such apples was recorded, and even close to 100.0% in some combinations (Tables 6–7).

Similar results with mechanical flower thinning and fruitlet thinning by hand, and also with the combined use of these two methods of regulating the fruiting of the Gala Mondial apple had been obtained by Peifer et al. [2018]. In their study, these authors obtained a similar percentage of yield reduction, and also an increase in fruit size. Beber et al. [2016] had found in their study that mechanical thinning of apple blossoms with additional hand thinning of fruitlets could be an effective method of ensuring optimal annual fruiting of apple trees.

Some of the thinning treatments in our experiment gave an undesirable result due to the development of exceptionally large apples. This was particularly evident in the combinations where one method of thinning was later supplemented by another. In the combinations M+H, M+C, C+H, and also H, the percentage of apples with a diameter of at least 8.0 cm and larger was from 12.4% to as high as 41.9%. In most of these combinations, the percentage of such large fruits was about 30.0% (Tables 5–7).Very large apples are more susceptible to bitter pit and a number of other diseases that are promoted by the low calcium content in the fruit, which may also reduce their storage life [Wójcik et al. 2009].

In the control combination (52.4 to 53.7%) and where only chemical thinning of fruitlets was performed, as much as 45.3 to 58.2% of the fruit developed colour on only up to 50% of the surface, whereas in the combination where hand thinning was the only treatment, the percentage of such fruit was only from 5.5 to 9.2%. A relatively small percentage (7.0 to 20.4%) of fruits with poorly developed colour on up to 50% of the surface was also obtained in the M+H combination (Tables 8–10).The highest number of well-coloured apples, with red blush coverage exceeding 75% of the skin surface, was obtained in the H and M+H combinations. In these combinations, the percentage of the most extensively coloured fruit ranged from 42.6 to 68.5% (Tables 8–10).

For comparison, in the experiment by Seehuber et al. [2014], the mechanical thinning with the BAUM device resulted in the knocking down of 25–33% of flowers from the trees. The mechanical thinning of apple blossoms was then supplemented by chemical or hand thinning of fruitlets, and the combined treatments contributed to improving the quality of the fruit. Apples from the trees subjected to thinning were larger, better coloured, and had a higher soluble solids content. The results obtained in our experiment are consistent with those of Seehuber et al. [2014].

In another study, Solomakhin and Blanke [2010] had mechanically thinned out flowers of the apple cultivars Golden Delicious Reinders and Gala Mondial with the aim of improving fruit quality and reducing the labour input for subsequent chemical and hand thinning of fruitlets. The control consisted of unthinned trees or trees thinned only by hand. The mechanical thinning of flowers had a positive effect on fruit size, firmness and soluble solids content compared with the fruit from the control trees.

In our experiment, results similar to those of Solomakhin and Blanke [2010] were obtained only in the improvement of fruit size. We found no significant differences in the firmness or the soluble solids content of the fruits from mechanically thinned trees in comparison with the control ones. In our study, apples from the trees subjected to any method of flower and/or fruit thinning, except for mechanical flower thinning alone and chemical of fruitlets in year 2022, had a significantly higher soluble solids content than those from the control trees (Tables 11–13).

Results similar to those of our experiment had been obtained in studies by other authors, such as Solomakhin et al. [2012] and Pflanz et al. [2016]. They found an improvement in fruit size, better colour development, and

Table 5. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the percentage of apples in different size grades in 2022

Treatments	Percentage of apples in size grades					
	6.0 cm	6.5 cm	7.0 cm	7.5 cm	8.0 cm	8.5 cm
Control	8.1 ±3.01 b*	20.4 ±1.58 d	34.7 ±2.87 cd	34.7 ±4.37 a	1.9 ±1.32 a	0.2 ±0.75 ab
M	0.0 ±0.00 a	1.2 ±0.65 b	20.2 ±3.24 bc	56.2 ±1.47 c	20.4 ±2.69 c	2.0 ±1.11 bc
M+H	0.0 ±0.00 a	0.1 ±0.50 ab	8.5 ±1.35 a	49.0 ±3.92 bc	37.5 ±3.68 de	4.9 ±0.87 cd
M+C	0.0 ±0.00 a	0.1 ±0.50 ab	15.1 ±2.48 ab	41.2 ±1.97 ab	32.2 ±1.44 cde	11.4 ±0.63 d
C	0.0 ±0.00 a	7.9 ±1.26 c	38.3 ±3.67 d	46.1 ±2.74 abc	7.7 ±1.32 b	0.0 ±0.00 a
C+H	0.0 ±0.00 a	0.2 ±0.75 ab	8.1 ±2.90 a	37.1 ±4.56 ab	45.8 ±3.11 e	8.8 ±3.84 d
H	0.0 ±0.00 a	0.0 ±0.00 a	24.2 ±4.17 bcd	46.3 ±3.49 abc	27.0 ±2.47 cd	2.5 ±1.08 bc

* For explanations, see Table 2.

Table 6. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the percentage of apples in different size grades in 2023

Treatments	Percentage of apples in size grades					
	6.0 cm	6.5 cm	7.0 cm	7.5 cm	8.0 cm	8.5 cm
Control	15.9 ±3.71 b*	43.9 ±3.38 c	34.9 ±4.39 bc	5.2 ±1.29 a	0.1 ±0.25 a	0.0 ±0.00 a
M	0.4 ±0.29 a	12.2 ±3.34 b	52.3 ±4.79 c	32.5 ±4.14 b	2.5 ±2.25 ab	0.1 ±0.25 ab
M+H	0.0 ±0.00 a	0.9 ±0.87 a	19.6 ±3.94 ab	47.9 ±2.25 bcd	26.7 ±3.16 c	4.9 ±2.17 c
M+C	0.0 ±0.00 a	6.9 ±3.22 b	39.0 ±2.72 bc	41.7 ±4.23 bc	11.7 ±3.33 bc	0.7 ±0.71 ab
C	14.1 ±1.35 b	46.1 ±3.33 c	33.4 ±3.90 bc	6.4 ±1.55 a	0.0 ±0.00 a	0.0 ±0.00 a
C+H	0.0 ±0.00 a	0.7 ±0.75 a	12.1 ±4.03 a	54.7 ±4.07 cd	30.8 ±2.71 c	1.7 ±0.71 bc
H	0.0 ±0.00 a	0.1 ±0.50 a	9.1 ±1.87 a	57.6 ±3.42 d	29.5 ±3.57 c	3.7 ±1.03 c

* For explanations, see Table 2.

Table 7. Effects of flower/fruitlet thinning of 'Gala Must'/M.9 trees on the percentage of apples in different size grades in 2024

Treatments	Percentage of apples in size grades					
	6.0 cm	6.5 cm	7.0 cm	7.5 cm	8.0 cm	8.5 cm
Control	11.6 ±2.97 b*	33.2 ±1.70 d	34.0 ±3.15 c	20.0 ±2.50 a	1.1 ±0.71 a	0.1 ±0.25 ab
M	0.2 ±0.00 a	7.2 ±2.96 c	33.7 ±1.44 c	45.5 ±2.02 b	12.2 ±0.85 b	1.2 ±0.63 bc
M+H	0.0 ±0.00 a	0.6 ±0.48 a	14.7 ±4.06 a	49.8 ±3.33 b	29.9 ±2.61 cd	5.0 ±1.18 d
M+C	0.0 ±0.00 a	3.8 ±1.38 bc	25.6 ±3.12 bc	41.3 ±3.04 b	22.9 ±3.52 c	6.4 ±0.48 d
C	7.4 ±0.65 b	26.8 ±2.25 d	36.0 ±2.17 c	26.1 ±0.50 a	3.7 ±0.65 a	0.0 ±0.00 a
C+H	0.0 ±0.00 a	0.9 ±0.25 ab	11.1 ±2.06 a	46.1 ±4.03 b	36.3 ±3.81 d	5.6 ±2.29 d
H	0.0 ±0.00 a	0.1 ±0.25 a	17.1 ±3.81 ab	49.7 ±2.33 b	29.7 ±2.17 cd	3.4 ±1.03 cd

* For explanations, see Table 2.

Table 8. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the percentage of apples in different blush coverage ranges in 2022

Treatments	Percentage of apples in blush coverage ranges			
	>25%	25–50%	50–75%	<75%
Control	10.8 ±1.55 c*	41.8 ±6.89 b	32.4 ±3.09 abc	15.0 ±6.30 a
M	3.9 ±1.26 b	24.6 ±6.29 b	41.9 ±3.30 bc	29.6 ±4.29 ab
M+H	0.0 ±0.00 a	7.0 ±2.90 a	24.5 ±2.47 a	68.5 ±3.20 c
M+C	1.1 ±1.15 ab	24.6 ±4.48 b	46.4 ±3.71 c	27.9 ±3.12 ab
C	2.3 ±1.25 b	43.0 ±3.38 b	38.4 ±5.06 bc	16.3 ±3.64 a
C+H	0.9 ±1.44 ab	25.0 ±3.57 b	34.0 ±5.89 abc	40.1 ±3.28 b
H	0.0 ±0.00 a	5.5 ±2.81 a	30.0 ±3.49 ab	64.5 ±5.85 c

* For explanations, see Table 2.

Table 9. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the percentage of apples in different blush coverage ranges in 2023

Treatments	Percentage of apples in blush coverage ranges			
	>25%	25–50%	50–75%	<75%
Control	7.4 ±1.47 b*	46.3 ±4.77 d	38.4 ±2.06 bc	7.9 ±2.50 a
M	0.4 ±0.29 a	24.1 ±5.20 bc	41.8 ±2.75 c	33.7 ±2.74 cd
M+H	1.1 ±0.41 a	19.3 ±4.01 ab	37.0 ±3.77 bc	42.6 ±3.94 de
M+C	2.9 ±1.75 ab	38.0 ±2.40 cd	37.1 ±1.60 bc	22.0 ±4.11 bc
C	6.4 ±1.70 b	51.8 ±4.66 d	28.7 ±2.53 a	13.1 ±3.51 ab
C+H	2.5 ±1.08 ab	27.2 ±3.04 bc	37.7 ±3.25 bc	32.6 ±4.44 cd
H	0.5 ±0.58 a	8.7 ±4.09 a	31.8 ±2.33 ab	59.0 ±5.76 e

* For explanations, see Table 2.

Table 10. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on the percentage of apples in different blush coverage ranges in 2024

Treatments	Percentage of apples in blush coverage ranges			
	>25%	25–50%	50–75%	<75%
Control	9.2 ±1.03 c*	43.2 ±2.78 c	35.4 ±1.63 ab	12.2 ±2.72 a
M	2.4 ±0.48 b	24.9 ±2.32 b	40.9 ±1.55 b	31.8 ±1.19 b
M+H	0.5 ±0,25 a	13.5 ±2.72 a	31.4 ±1.04 a	54.6 ±4.03 c
M+C	2.6 ±0.85 b	31.0 ±2.25 b	41.3 ±2.17 b	25.1 ±3.25 b
C	4.7 ±1.55 b	46.5 ±2.59 c	33.6 ±2.25 a	15.2 ±1.08 a
C+H	2.4 ±1.08 b	26.4 ±3.75 b	35.9 ±4.03 ab	35.3 ±3.99 b
H	0.3 ±0.29 a	8.0 ±1.26 a	31.1 ±0.75 a	60.6 ±1.03 c

* For explanations, see Table 2.

Table 11. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on fruit firmness and soluble solids content immediately after harvest, and the mean number of apples per tree in 2022

Treatments	Firmness (kG)	Soluble solids (%)	Mean number of apples (per tree)
Control	7.9 ±0.11 a*	13.0 ±0.34 a	298 ±0.33 c
M	8.0 ±0.11 a	13.3 ±0.31 a	204 ±6.64 b
M+H	8.7 ±0.18 c	14.4 ±0.33 bc	110 ±4.62 a
M+C	8.8 ±0.20 c	14.8 ±0.40 c	98 ±1.15 a
C	8.5 ±0.11 bc	13.7 ±0.30 ab	276 ±8.95 c
C+H	9.4 ±0.17 d	15.7 ±0.37 d	92 ±3.76 a
H	8.2 ±0.24 ab	15.0 ±0.26 cd	131 ±4.00 a

* For explanations, see Table 2.

Table 12. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on fruit firmness and soluble solids content immediately after harvest, and the mean number of apples per tree in 2023

Treatments	Firmness (kG)	Soluble solids (%)	Mean number of apples (per tree)
Control	6.5 ±0.12 ab*	12.5 ±0.28 a	537 ±5.20 d
M	6.3 ±0.15 a	12.6 ±0.30 a	383 ±10.11 c
M+H	6.7 ±0.11 abc	15.3 ±0.21 d	181 ±8.66 a
M+C	6.5 ±0.18 ab	14.5 ±0.20 c	273 ±10.39 b
C	6.4 ±0.15 a	13.4 ±0.30 b	452 ±7.80 c
C+H	6.9 ±0.11 bc	14.8 ±0.16 cd	210 ±4.62 ab
H	7.0 ±0.15 c	14.4 ±0.26 c	217 ±3.46 ab

* For explanations, see Table 2.

Table 13. Effects of flower/fruitlet thinning of Gala Must/M.9 trees on fruit firmness and soluble solids content immediately after harvest, and the mean number of apples per tree in 2024

Treatments	Firmness (kG)	Soluble solids (%)	Mean number of apples (per tree)
Control	7.2 ±0.08 bc*	12.7 ±0.21 a	418 ±6.93 e
M	7.1 ±0.11 b	12.9 ±0.22 a	294 ±8.66 c
M+H	6.7 ±0.10 a	15.3 ±0.21 d	146 ±6.93 a
M+C	7.6 ±0.12 d	14.7 ±0.13 c	190 ±6.93 b
C	7.4 ±0.10 cd	13.5 ±0.23 b	366 ±8.08 d
C+H	7.1 ±0.11 b	15.3 ±0.23 d	154 ±8.08 ab
H	7.0 ±0.13 ab	14.7 ±0.15 c	168 ±4.04 ab

* For explanations, see Table 2.

higher soluble solids content in apples collected from the trees on which mechanical flower thinning had been performed with the BAUM device, compared with unthinned trees. Schupp and Kon [2014], in turn, reported that in their experiment they found no differences in soluble solids content in the fruits harvested from those trees on which flowers had been mechanically thinned out, compared with the fruits from only hand-thinned trees.

In another study, Win et al. [2023] subjected Fuji apple trees to mechanical thinning of flowers with the Darwin device and to chemical thinning of fruitlets, and also to the combined use of these two thinning methods, and compared the results with hand thinning as the control. The authors found that none of the thinning methods had a significant effect on fruit size, weight, or colour. However, the treatments improved fruit firmness and soluble solids content immediately after harvest.

In a study by Misimović et al. [2012], apple fruitlets were thinned out by means of the natural foliar fertilizers Goëmar BM 86 E (a product from algae GA14 Ascophyllum nodosum + N, MG and Mo) and Goëmar Folical (GA14 + Ca and B). The authors found that after spraying apple trees with these fertilizers, the shedding of fruitlets increased, compared with the control trees. The harvested fruits had a greater weight and contained more soluble solids as a result of foliar fertilization, but were less firm than apples from the control combination.

Schupp and Kon [2014] found that mechanical flower thinning increased apple firmness, relative to the control. Unlike the results obtained by Solomakhin and Blanke [2010] and Schupp and Kon [2014], the results for fruit firmness in our study were not unambiguous. In one year, some flower and/or fruit thinning treatments significantly increased the firmness of apples compared with those harvested from the control trees, while in another year the firmness of such fruit was lower or no significant differences were found (Tables 11–13).

All of the flower and/or fruit thinning methods used, except for the chemical thinning of fruitlets with the preparation Globaryll in 2022, significantly reduced the number of fruits per tree compared with the control (Tables 11–13). It seems that the flowers/fruitlets were thinned out too much, especially in the M+H and C+H combinations, and where the fruitlets were only thinned out by hand. As a result, a large percentage of the fruit harvested from the trees in these combinations, in the size range of 8.0 cm and larger, were apples that were evidently too large. Such large apples are susceptible to many diseases and may not keep well.

Lordan et al. [2018] in their study report that reducing the rotational speed of the rotor that removes flower buds in the Darwin machine from 270 to 230 rpm, at a tractor speed of 5 km·h^{-1}, helped to obtain the optimal number of fruits per tree. Using these parameters of the tractor and the mechanical flower thinning machine, the authors did not achieve significant differences in the yield and size of apples of the Gala in comparison with hand or chemical thinning. The results obtained by Lordan et al. [2018] were consistent with those of Seehuber et al. [2014], who also found that reducing the speed of the rotors reduced the thinning effect.

In our study, significant differences in fruit yield and size were noted mainly where fruitlets had been thinned out by hand, compared with the chemical and mechanical thinning of flower buds. In another study by Solomakhin et al. [2012] conducted on Golden Reinders® apple trees, it was concluded that no significant differences in fruit yield were observed when comparing hand thinning of fruitlets with mechanical thinning of flower buds at a tractor speed of 5–7.5 km·h^{-1} and 300–480 rpm of the rotors. The study conducted by Veal et al. [2011] had suggested that to obtain the best effectiveness of mechanical flower thinning in the cultivars Golden Delicious, Gala, Elstar and Braeburn, a tractor speed of 5–7.5 km·h^{-1} and rotor speed of 300–420 rpm were needed.

CONCLUSIONS

1. With the exception of the mechanical thinning of flowers and chemical thinning of fruitlets, all other methods of thinning caused a significant reduction in apple yield, and only with the exception of the chemical thinning of fruitlets did they significantly increase mean fruit weight.
2. The best results of the thinning were found as a result of mechanical thinning of flowers with the BAUM device, reducing fruit yield by 7.2 to 17.5%, depending on the year and caused increase of the number of fruits within the desired marketable size range of 7.0–7.5 cm in diameter.
3. Apples from trees mechanically thinned using the BAUM device had a higher average fruit weight than those from chemically thinned trees, but lower

than those from manually thinned trees, and also had a lower soluble solids concentration than fruit from both of these treatments.

4. Chemical thinning of fruitlets reduced fruit yield by 7.5 to 12.3%, depending on the year, and resulted in the production of a large number of undesirably small apples.
5. Combining the different methods of flower and fruitlet thinning, as well as thinning by hand only, resulted in excessive growth of apples to a diameter of 8.0 cm and above.
6. Most of the flower and/or fruitlet thinning treatments increased the soluble solids content relative to its level in the control fruit.

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EFFECT OF CASING SOIL TYPE AND HUMIDITY ON GINGER BLOTCH DEVELOPMENT IN MUSHROOM CULTIVATION

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ABSTRACT

Pseudomonas 'gingeri' is the cause of ginger blotch disease of the white button mushrooms (*Agaricus bisporus*). The occurrence of the disease in the cultivation results in the appearance of ginger discolouration on the mushroom caps. Currently, there is no effective method of protecting the mushroom from bacterial infection. Therefore, the selection of appropriate substrates for mushroom cultivation and environmental cultivation conditions, such as relative humidity, are of high importance in controlling the disease. The aim of the study was to evaluate the effect, on the development of ginger blotch, of two types of peat-based casing soil, with different water holding capacity, and two different air relative humidities inside the mushroom growing chamber. The cultivation trials were artificially infected with two *P. 'gingeri'* isolates, at two different inoculation doses. The blotch disease incidence on the heavy casing soil, which characterised lower water holding capacity, was significantly higher than on the medium one, regardless of the number of bacterial cells and bacterial isolate. The results also demonstrated a significant correlation between higher levels of air humidity (90% in the cultivation chamber) and the ginger blotch prevalence. It was determined that the type of casing soil and the level of air humidity in the mushroom growing room are of crucial importance for efficient mushroom cultivation. These factors can also play a significant role in preventing against bacterial disease development.

Keywords: *Agaricus bisporus*, bacterial disease, crop conditions, cultivated mushrooms, *Pseudomonas 'gingeri'*

INTRODUCTION

The white button mushroom, *Agaricus bisporus*, is the most commonly cultivated mushroom around the world. For a many years, Poland has been the largest producer of mushrooms in Europe, as well as the largest global exporter of fresh mushrooms [Siwulski et al. 2022]. The cultivation of mushrooms is undertaken in conditions characterised by high levels of air humidity and temperature. The use of a limited range of plant protection products in mushroom cultivation increases the risk of bacterial infections [Gea et al. 2021]. The process of cultivating white button mushrooms involves the use of two substrates, i.e. compost colonised with mycelium and the layer of peat casing. This is essential to stimulate and enhance the development of mushroom sporophores [Noble et al. 2003, Pardo et al. 2002]. Peat casing soils that are available for use in the cultivation of mushrooms vary in their physicochemical properties, particularly with regard to their capacity for water retention, maximum humidity, pH level and ash content. The selection of an appropriate type of peat casing is as important as the high quality of the compost and the

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maintenance of the cultivation conditions [Dias et al. 2021, Noble et al. 2024].

Both media used in the cultivation of mushrooms are colonized by a broad range of bacteria, archaea and fungi. Among identified bacteria, some members of the genus *Pseudomonas* appear to be associated with the development of *A. bisporus*, i.e. *P. putida* [McGee 2018], but other with bacterial disease incidence, i.e. *P. 'gingeri'* or *P. tolaasii* [Paine 1919, Braat et al. 2022, Fletcher and Gaze 2008]. Bacterial diseases are responsible for discolouration and deformation of the mushroom caps. This has resulted in significant economic losses caused by reduced crop yield (i.e. pinhead death), deterioration in mushroom quality, and a reduction in shelf life post-harvest [Sapers et al. 2001].

Pseudomonas 'gingeri' was first reported to cause yellow-brown spots on *A. bisporus* in the UK in 1982 [Wong et al. 1982]. These blotch symptoms are only superficial and do not result in the formation of depressions on the caps. However, in the case of high severity of the disease, the fruiting bodies are susceptible to damage, with spots developing on the edges of the caps and eventually covering their entire surface [Soler-Rivas et al. 1999]. Gandy [1967] demonstrated a direct correlation between the development of the bacterial disease and the concentration of pathogen cells in the casing soil. Conversely, the findings reported by Taparia et al. [2021a] suggest that the symptoms exhibited by the disease may not be exclusively attributable to the presence of bacteria in the casing soil.

Therefore, it is challenging to establish a clear reason for the occurrence of bacterial disease in a crop. It appears that the peat casing may be a primary source of bacteria, but it does not appear to be the only source of infection. The transmission of bacteria occurs through exposure to contaminated environments, such as infected mushroom farms, equipment or infected fruiting bodies, through direct contact with workers [Wong and Preece 1982, Mamoun et al. 1999]. The literature suggests that a small number of bacteria in the casing soil can cause disease symptoms, however, introducing a particular number of cells into the casing soil does not always cause blotch symptoms on the mushrooms. Consequently, the occurrence of bacterial infection is largely caused by the crop conditions in the growing mushroom chamber, methods of cultivation, mushroom strain or other factors, such as the type of casing soil used in the cultivation [Moquet et al. 1996, Soler-Rivas et al. 1999, Szumigaj-Tarnowska and Uliński 2022].

The aim of the present study was to determine the effect of two types of peat-based casing soil and air humidity on the severity of ginger blotch symptoms caused by two *P. 'gingeri'* isolates, at two different inoculation doses.

MATERIAL AND METHODS

Bacterial isolates

In the present study, two isolates of *P. 'gingeri'* bacteria, refereed to MO and B7, were selected for experimental analysis. Isolates were obtained from mushrooms exhibiting symptoms of ginger blotch, originating from Polish mushroom farms. The species of the isolates was determined by conducting a comprehensive biochemical, molecular, and pathogenicity test. The identification process involved a detailed macroscopic and microscopic evaluation, in addition to biochemical tests, including API 20NE tests. These tests involved: Gram staining of bacteria [Beveridge 2001], the ability to produce fluorescent pigment [King et al. 1954] and the type of glucose metabolism [Hugh and Leifson 1953]. In addition, the activity of oxidase, catalase, the ability of the bacteria to hydrolyze gelatin and starch, to decompose nitrates, and to asimilate carbon from citrate [Lelliott et al. 1966] were examined. According to the key to bacterial identification [Buchanan and Gibbons 1974], the isolates were classified into the appropriate genus. In the final stage of identification, commercial API 20NE tests were performed, and a biotest of pathogenicity on mushroom tissue was used to confirm the pathogenicity of the isolates [Taparia et al. 2021b]. The experiments used 24-hour bacterial cultures activated at 24°C in Nutrient Broth liquid medium.

Cropping trials

The study involved a pair of distinct experiments. The initial experiment examined the impact of two types of peat casing, while the second study focused on the effect of relative air humidity, on the development of ginger blotch. The experiments were carried

out within controlled conditions in the growing house chambers in 22 cm diameter pots (surface area was 0.04 m^2) filled with 1.7 kg of phase III compost fully colonized with the *A. bisporus* strain Triple X (Sylvan).

The surface of the compost was then covered with a 4 cm peat casing layer. Two types of peat casing soil (hereafter refereed as heavy and medium casing) with different physicochemical properties (Table 1) were obtained from a local producer of peat casing for mushroom cultivation, which was located in Skierniewice, Poland. Casing soils had been prepared especially for the experimental research. The conditions in the mushroom growing chamber while incubation phase were maintained as in routine production, i.e., the temperature was 23–24°C, carbon dioxide concentration was 3000 mg L^{-1}, and relative humidity was 95%. After seven days, when the mycelium reached the surface of the casing, the temperature was lowered to 18°C, and the carbon dioxide concentration was reduced to 1000–1200 mg L^{-1}. Then the casing surface was sprayed with 10 ml of bacterial cells suspension, with different inoculum levels being used to achieve a population density of either 2.6×10^8 or 2.6×10^7 cfu m^{-2} of casing. Control trials were not infected by *P. 'gingeri'* cells [Wong and Preece 1982]. The crop was watered for four days at 10 L m^{-2} before the first flush and 8 L m^{-2} before the second flush. The amount of water used was calculated based on the amount of substrate per m^2 and its moisture level. It was determined that the optimal water dosage is approximately 20 L m^{-2}, assuming a substrate density of 90 kg m^{-2} and a humidity level of 64%. During the growth of the fruiting bodies and harvesting, the temperature in the growing chamber was maintained at 17.8 ±1.0°C, the carbon dioxide concentration was 1100–1200 mg L^{-1} and the relative humidity was 88%. The temperature of the substrate was 1.0–1.5°C higher than the air temperature, and no overheating of the mushroom substrate and peat casing was observed. The use of different peat casings did not affect the temperature in the casing layer.

In the study of the effect of relative humidity on the development of ginger blotch, a mixed peat was used as casing soil. Following the application of the peat casing, the crop was infected with a bacterial suspension, using the same methodology as in the experiment with different casing soils. During the growth of the fruiting bodies and harvesting, the temperature in the growing chamber was regulated to 17.8 ±1.0°C, the carbon dioxide concentration to 1100–1200 mg L^{-1} and the relative humidity to 86% or 90%, depending on the variant tested. The development of ginger blotch was assessed based on the yield of healthy and diseased mushrooms. The disease severity index (DSI) in the first and second flush of the mushroom was determined as the ratio of the yield of infected fruiting bodies to the total yield obtained in a given combination according

Table 1. The physicochemical properties of the peat casing soils used in the experiments

Type of peat casing soil	Measured parameters					
	bulk density (g mL^{-1})	water capacity** (%)	maximum humidity*** (%)	dry bulk density (g mL^{-1})	pH	ash (%)
Heavy peat casing soil with a predominance of low peat*	1.186	88.1	74.3	0.304	7.7	71.8
Medium peat casing soil from a mixture of low and high peat	1.09	92.2	84.8	0.185	7.6	47.3

* Peat soils are commonly used as a casing layer: low peat is characterized by a lower organic matter content compared to high peat and a lower water retention capacity; high peat contains a higher percentage of organic matter and is more fibrous. Its ability to hold water helps maintain a moisture environment for mushroom growth.

** water capacity (%) – ability of casing to absorb, retain and gradually release water to establish an optimal environment for mushroom growth; it plays a crucial role in maintaining humidity, aeration and microbial activity for appropriate mushroom growth

*** maximum humidity (%) – maximum water content that the casing can reach before becoming oversaturated and negatively affecting mushroom growth

Table 2. Average yield of healthy and infected mushrooms according to the number of *Pseudomonas 'gingeri'* cells and peat casing type over two flushes (1st experiment)

Number of bacteria cells (cfu m^{-2})		1st flush		2nd flush	
		type of peat casing soil			
		heavy	medium	heavy	medium
Yield of healthy mushrooms (kg m^{-2})					
Control (non-inoculated)		23.25 ±3.62 Aa	22.29 ±4.29 Aa	9.25 ±1.43 Aa	10.87 ±1.25 Aa
MO	2.6×10^8	7.45 ±0.79 Bc	13.56 ±2.24 Ac	4.73 ±1.22 Bb	7.78 ±2.61 Ab
	2.6×10^7	8.35 ±1.13 Bbc	19.32 ±2.51 Ab	6.06 ±2.15 Bb	8.35 ±2.33 Ab
B7	2.6×10^8	11.73 ±2.32 Bb	14.92 ±2.83 Ac	6.68 ±1.38 Ab	7.51 ±1.52 Ab
	2.6×10^7	10.11 ±1.73 Bbc	19.60 ±3.32 Ab	9.65 ±2.02 Aa	7.80 ±2.13 Ab
Mean		12.18 ±6.40 B	17.94 ±3.60 A	7.27 ±2.11 B	8.46 ±1.38 A
Yield of infected mushrooms (kg m^{-2})					
Control (non-inoculated)		0.0 ±0.0 Ab	0.0 ±0.0 Ac	0.0 ±0.0 Ab	0.0 ±0.0 Ab
MO	2.6×10^8	14.38 ±2.03 Aa	7.71 ±2.32 Ba	6.24 ±1.72 Aa	4.23 ±1.83 Aa
	2.6×10^7	15.38 ±3.23 Aa	2.93 ±0.85 Bb	2.21 ±0.93 Ab	1.11 ±0.86 Ab
B7	2.6×10^8	12.82 ±2.75 Aa	5.81 ±1.05 Bab	2.69 ±1.01 Ab	1.75 ±0.96 Ab
	2.6×10^7	13.18 ±3.28 Aa	3.27 ±1.28 Bb	2.83 ±1.31 Ab	1.05 ±0.69 Bb
Mean		11.15 ±6.32 A	3.94 ±2.94 B	2.79 ±2.24 A	1.63 ±1.58 B

Values are means of four samples ±standard deviation (SD); means in the same row, for each flush, with the same capital letter do not differ statistically ($P < 0.05$, Newman-Keuls test); means in the same column, within the particular yield, with the same lowercase letter do not differ statistically ($P < 0.05$, Newman-Keuls test)

to the formula: NC (%) = (Poc / Pc) × 100%, where: Poc – yield of diseased fruiting bodies, Pc – total yield (yield of healthy and diseased fruiting bodies).

Statistical methods

The experiments were conducted twice, with four replicates each. Each experiment had three distinct factors: the first factor was the type of peat casing soil or air humidity, the second was the bacterial isolate, and the third was the bacterial inoculum. The statistical analysis was conducted using the analysis of variance, and the differences between the means were compared according to the Newman-Keuls test at a significance level of $P < 0.05$. To evaluate the relationship between the disease severity and the type of peat casing soil or relative humidity, a t-Student test was used with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Effect of different type of peat casing soil

The present study examined the prevalence of ginger blotch disease in relation to two different types of casing soils. The peat casing soils used differed in the following parameters: bulk density, water capacity and maximum humidity. It was found that the physical and chemical characteristics of the casing soil can determine the growth of mushroom mycelia and yield, or can even influence the susceptibility of mushrooms to infection by pathogens and the severity of the disease [Gea et al. 2013].

In the first experiment (Table 2) the infection of the crop by *P. 'gingeri'* caused a significant reduction in the yield of healthy mushrooms in the first flush on heavy casing soil, with the average yield of

Table 3. Average yield of healthy and infected mushrooms according to the number of *Pseudomonas 'gingeri'* cells and peat casing type over two flushes (2nd experiment)

Number of bacteria cells (cfu m^{-2})		Yield of healthy mushrooms (kg m^{-2})			
		1st flush		2nd flush	
		type of peat casing soil			
		heavy	medium	heavy	medium
Control (non-inoculated)		23.31 ±4.21 Aa	24.65 ±3.52 Aa	16.82 ±1.95 Aa	16.53 ±1.64 Aa
MO	2.6×10^8	20.18 ±4.15 Aab	20.60 ±4.26 Aa	9.89 ±1.31 Abc	11.33 ±2.50 Ab
	2.6×10^7	21.50 ±4.39 Aa	19.90 ±3.87 Aab	12.73 ±2.58 Ab	13.08 ±3.43 Aab
B7	2.6×10^8	18.19 ±3.36 Ab	16.95 ±3.74 Ab	8.08 ±2.25 Bc	13.48 ±3.27 Aab
	2.6×10^7	21.76 ±4.29 Aa	19.87 ±4.16 Aab	7.89 ±1.30 Bc	12.58 ±2.38 Aab
Mean		20.99 ±1.92 A	20.39 ±2.76 A	11.08 ±3.75 B	13.40 ±1.93 A
		Yield of infected mushrooms (kg m^{-2})			
Control (non-inoculated)		0.0 ±0.0 Ab	0.0 ±0.0 Ac	0.0 ±0.0 Ac	0.0 ±0.0 Ab
MO	2.6×10^8	5.81 ±1.38 Aa	3.35 ±3.39 Ab	2.26 ±1.12 Ab	1.88 ±1.31 Ab
	2.6×10^7	1.35 ±0.85 Ab	2.15 ±1.26 Abc	1.02 ±0.93 Abc	0.0 ±0.0 Ab
B7	2.6×10^8	8.04 ±1.52 Aa	6.67 ±2.35 Aa	6.95 ±2.26 Aa	5.08 ±2.13 Aa
	2.6×10^7	0.92 ±0.55 Ab	1.65 ±1.03 Abc	4.93 ±1.73 Aa	2.52 ±1.28 Bb
Mean		3.22 ±3.50 A	2.76 ±2.47 A	3.03 ±2.81 A	1.90 ±2.10 B

Explanations – see Table 2.

12.18 kg m^{-2}. The average yield of healthy fruiting bodies (17.94 kg m^{-2}) on the medium casing soil was significantly higher than the yield on the heavy one. A similar relationship was obtained in the second flush, as the average yield of healthy sporophores on medium casing soil was 8.46 kg m^{-2} and was significantly higher than on heavy casing. Furthermore, in the first and second flush, the average yield of infected mushrooms on the heavy casing soil was significantly higher than on the medium.

In the second experiment the yield of healthy and diseased mushrooms in the first flush showed no significant difference between the casing soils that were tested (Table 3). In the second flush, a significantly higher average yield of healthy sporophores was found on the medium casing soil, which amounted to 13.40 kg m^{-2}, as opposed to the heavy one. The yield of infected mushrooms was significantly lower on the medium casing than on the heavy one.

According to Navarro et al. [2021] the appropriate physicochemical parameter of casing soil used in the mushroom cultivation has a great influence on the yield. The casing soils used in the study differed in their water holding capacity. In the case of the use of casing soil with a lower water holding capacity (i.e. heavy casing), the ability of the casing soil to absorb water is reduced. Consequently, after watering the crop, the casing layer can lead to the development of moist conditions, resulting in an increase in moisture within the casing and difficulty in evaporation of moisture from the mushroom surface. Such conditions are conducive to the development of bacterial diseases [Lomax 2007, Navarro et al. 2018, Navarro et al. 2021]. Taparia et al. [2021b] also demonstrated that the prevalence of ginger blotch varied according to the type of casing soil. Gea et al. [2013] observed that he incidence of dry bubble disease, caused by the fungus *Lecanicillium fungicola*, was more frequent on casing

Table 4. Statistical analysis of the relationship between the ginger blotch severity (%) and the type of peat casing soils in two flushes

Parameter	1st flush		2nd flush	
	heavy casing	medium casing	heavy casing	medium casing
Mean	38.69 A	18.79 B	34.34 A	14.74 B
Median	41.53	14.47	28.31	13.03
df	7	–	7	–
t Stat	4.946	–	12.782	–
Variation	406.67	–	59.067	–
SE	4.023	–	1.533	–
t-test ($p = 0.05$)	1.8945	–	1.8945	–

Means in the same rows, for particular flushes, with the same letter do not differ statistically ($P < 0.05$, *t*-Student test, n = 8).

soil, which was characterized by a lower water capacity, when compared to mineral soil with *Sphagnum* peat. Similar results were obtained by Carrasco et al. [2015] and Ślusarski et al. [2012], who studied the effect of different casing soils on the development of *Cladobotryum dendroides* in the mushroom cultivation. The casing layer with the lowest water holding capacity favored the development of the disease.

Regardless of the number of bacterial cells and bacterial isolate, an analysis of the relationship between the severity of ginger blotch (expressed as a percentage of the yield of diseased mushrooms) and the type of peat casing soils in two flushes is presented in Table 4. The results of analysis have demonstrated that in the first and the second flush the degree of disease incidence on the heavy casing was significantly higher than on the medium one. Furthermore, as shown in Figure 1, the values of bacterial blotch intensity obtained on the heavy casing were more dispersed than those obtained on the medium. A further analysis of the results revealed that on the heavy casing a greater frequency of disease incidence values were above

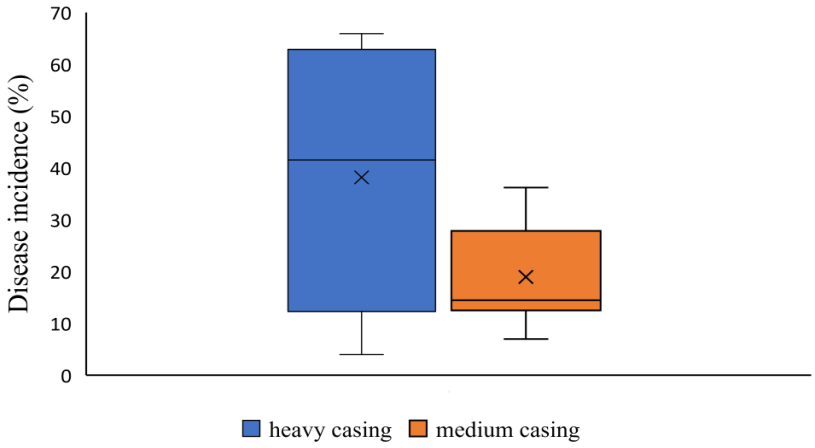

Fig. 1. Ginger blotch disease severity (%) according to the type of peat casing in the first flush in the mushroom cultivation infected with *P. 'gingeri'* cells (n = 8)

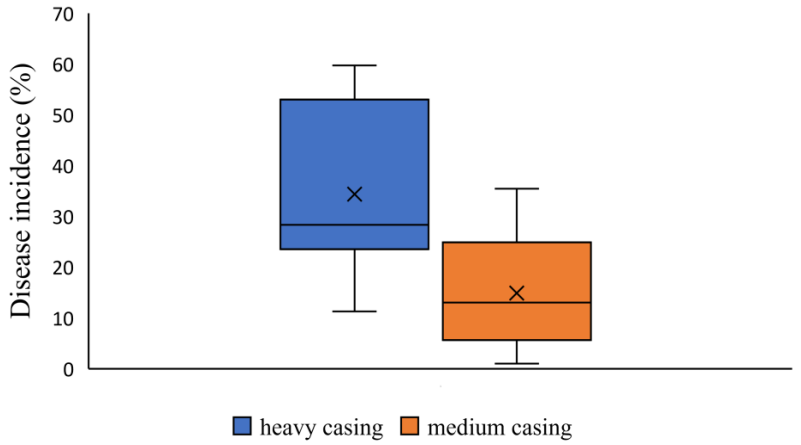

Fig. 2. Ginger blotch disease severity (%) according to the type of peat casing in the second flush in the mushroom cultivation infected with *P. 'gingeri'* cells (n = 8)

41.53%, while on the medium casing higher proportion of values were below 14.47% (Fig. 1). In the second flush on the heavy casing the disease symptom intensity levels were also higher than on the medium casing. What is more, the results indicated that on the heavy casing more values for disease incidence were below 28.31%, while on the medium casing the values were evenly spread around the median of 13.03% (Fig. 2).

Effect of relative humidity on bacterial blotch

Literature data suggest that the maintenance of adequate cultivation conditions can reduce the incidence of bacterial diseases [Mamoun et al. 1999, Navarro et al. 2018]. Moquet et al. [1996] found that the susceptibility of mushrooms to blotch disease is dependent on both the toxin secreted by the bacteria and other factors. The studies aimed to determine the effect of humidity on the development of bacterial disease. Analysis of the yield of healthy fruiting bodies in the control trials revealed no statistically significant differences between the humidity levels (Table 5). However, the infection by *P. 'gingeri'* isolates resulted in significant reduction in the yield of healthy fruiting bodies in the studied crops. The average yield of healthy mushrooms (16.17 kg m^{-2}) at 90% humidity was significantly lower than the yield (18.60 kg m^{-2}) at 86% humidity. In parallel the yield of infected fruiting bodies was found to be significantly higher at 90% humidity, reaching 6.33 kg m^{-2}, in contrast to the yield calculated at 3.24 kg m^{-2} at 86% humidity. Statistical analysis confirmed the interaction of two factors (number of bacterial cells and humidity) on the mushrooms yield. The remaining two-factor interactions (i.e. humidity and isolate, isolate and inoculum) and the three-factor interaction were found to be non-significant. In the second flush, the development of the bacterial disease was not observed, and the yield of healthy mushrooms did not differ between the variants of cultivation.

The bacterial blotch prevalence in relation to the number of bacteria cells and relative humidity is shown in Figure 3. It was shown that a higher number of bacterial cells (2.6×10^8 cfu m^{-2}) at 90% humidity resulted in a significant increase in disease incidence (DSI level was 40%) compared to 86% humidity (17%). The impact of humidity on the prevalence of bacterial diseases in the mushroom cultivation has been documented by Fletcher and Gaze [2008], and Navarro et al. [2018].

In the present study, no disease symptoms were observed in the second flush. A similar phenomenon was noted by Taparia et al. [2021b], who observed a reduction of ginger blotch disease symptoms in the second flush. In addition, Olivier et al. [1997] and Moquet et al. [1998] found that fruiting bodies from second flush exhibited higher resistance to bacterial blotch, as revealed by a reduced yield of mushrooms with disease symptoms.

Table 5. Average yield of healthy and infected mushrooms according to the number of *Pseudomonas 'gingeri'* cells and relative humidity over two flushes

Number of bacteria cells (cfu m^{-2})		Yield of healthy mushrooms (kg m^{-2})			
		1st flush		2nd flush	
		relative humidity			
		86%	90%	86%	90%
Control (non-inoculated)		20.36 ±4.31 Aa	21.75 ±4.88 Aa	8.42±2.32 Aa	9.31 ±2.37 Aa
MO	2.6×10^8	14.87 ±3.73 Ac	8.57 ±3.13 Bc	8.33 ±3.73 Aa	7.76 ±3.81 Aa
	2.6×10^7	17.83 ±4.37 Ab	18.82 ±3.43 Aa	8.50 ±4.13 Aa	8.25±3.39 Aa
B7	2.6×10^8	19.77 ±4.21 Aab	13.03 ±2.45 Bb	7.75 ±3.64 Aa	7.85 ±2.67 Aa
	2.6×10^7	20.18 ±3.66 Aa	18.68 ±2.50 Aa	8.36 ±2.88 Aa	8.11 ±3.10 Aa
Mean		18.60 ±2.32 A	16.17 ±5.29 B	8.27 ±0.30 A	8.26 ±0.62 A
		Yield of infected mushrooms (kg m^{-2})			
Control (non-inoculated)		0.0 ±0.0 Ac	0.0 ±0.0 Ad	0.0 ±0.0 A	0.0 ±0.0 A
MO	2.6×10^8	7.38 ±3.28 Ba	14.23 ±4.33 Aa	0.0 ±0.0 Aa	0.0 ±0.0 Aa
	2.6×10^7	3.53 ±2.16 Ab	3.40 ±2.15 Ac	0.0 ±0.0 Aa	0.0 ±0.0 Aa
B7	2.6×10^8	3.62 ±2.41 Bb	9.85 ±3.14 Ab	0.0 ±0.0 Aa	0.0 ±0.0 Aa
	2.6×10^7	1.65 ±1.08 Ab	4.18 ±2.82 Ac	0.0 ±0.0 Aa	0.0 ±0.0 Aa
Mean		3.24 ±2.75 B	6.33 ±5.66 A	0.0 ±0.0 A	0.0 ±0.0 A

Explanations – see Table 2.

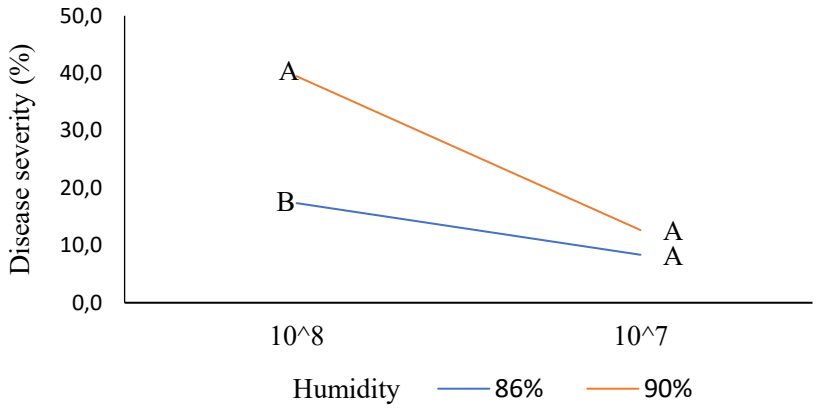

Fig. 3. Severity of ginger blotch (%) according to the relative humidity in the crop infected with *P. gingeri* at different number of cells. Means for the number of cells (i.e. 2.6×10^8 or 2.6×10^7 m^{-2}) with the same letter do not differ statistically ($P < 0.05$, *t*-Student test)

CONCLUSIONS

The present study has shown that the appropriate peat casing soil and air relative humidity levels in the mushroom growing chamber can influence of the reduction in bacterial blotch prevalence caused by *P. 'gingeri'*. Heavy casing soil used in the mushroom cropping trials, characterized by higher wet bulk density and maximum humidity as well as lower water capacity has been associated with an increase in the intensity of the ginger blotch disease. It was also revealed that higher levels of relative humidity in the cultivation chamber resulted in the manifestation of ginger blotch symptoms, particularly in the first flush. It was concluded that, in order to control of bacterial diseases in the mushroom cultivation, it would be necessary to use a mixed-peat casing soil, which would allow for the increased water capacity. Furthermore, an appropriate level of humidity in the growing chamber is crucial for efficient mushroom cultivation, as it can play an essential role in preventing the development of bacterial diseases.

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EFFECT OF DIFFERENT SOWING TIMES, PLANT DENSITIES, AND FERTILISER DOSES ON YIELD AND CROP ELEMENTS OF DRY BEAN VARIETIES (*Phaseolus vulgaris* L.)

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is the most widely consumed grain legume in Europe, yet dry bean production in Hungary remains below domestic demand. This study aimed to identify optimal cultivation strategies for improving yield by evaluating the combined effects of sowing time, plant density, and fertiliser dose across three dry bean varieties under field conditions in Nyíregyháza (Hungary) from 2015 to 2017. The results revealed that all three agronomic factors significantly influenced yield and its components, with varietal differences in response patterns. Sowing time and plant density consistently affected pod number, thou-sand-seed weight, and yield per plant. Fertiliser application had a more variable impact, de-pending on the variety and year. The findings underline the importance of variety-specific recommendations to optimise yield under varying environmental and soil conditions.

Keywords: agronomic factors, cultivation strategies, grain legume, yield elements, fertiliser

INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are important for their nutritional value and their ability to enhance soil fertility through nitrogen fixation [McKenzie et al. 2001, Kádár 2005]. In Hungary, domestic production remains insufficient to meet the increasing demand from consumers and the food industry. However, successful dry bean cultivation is highly sensitive to environmen-tal conditions, especially temperature and rainfall, which can negatively influence flowering and pod development [Ovacikli and Tolay 2020].

Although beans can fix atmospheric nitrogen through symbiosis with Rhizobium bacteria [Kádár 2005], this process only becomes effective at later growth stages. Therefore, supplying starter nitrogen is generally recommended to support early development [McKenzie et al. 2001]. Fertilisation with nitrogen, phosphorus, and potassium significantly increases yield and its key components, such as pod number, seeds per pod, thousand-seed weight, and total yield [Begum et al. 2003, Balláné Kovács 2011, Fageria et al. 2013, Faria and Fageria 2014, Mbeke et al. 2014, Turuko and Mohammed 2014, Fageria and Baligar 2016, Seif et al. 2016, Soratto et al. 2017]. Teferi et al. [2022] showed that increasing the N fertiliser dose improved pod number and yield per plant; however, doses exceeding the optimal level had adverse effects. The effects of fertilisation can differ significantly based on the genotype and the

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current cli-matic conditions [Fageria et al. 2001, Kádár 2013, Karavidas et al. 2022].

Plant density is a key factor influencing bean development. Lower plant densities promote branching and pod formation [Jan et al. 2002, Samago et al. 2018, Bakure et al. 2023]. In contrast, excessively high densities can reduce the yield per plant as competition for light, nutrients, and water increases [Shiv Kumar and Mishra 2002, Tuarira and Moses 2014, Ahmed et al. 2016]. The optimal plant density is not universal and often depends on varietal traits [Shirtliffe and Johnston 2002, Pawar et al. 2007, Mekonnen et al. 2012, Soratto et al. 2017]. Moniruzzaman et al. [2009] and Mehmet [2008] examined the effects of plant density and N fertiliser combination. The highest yield per plant, the most pods per plant, and the most considerable thousandseed weight were observed in the treatment with the lowest plant density and the highest nitrogen application. The highest yield per hectare was achieved at the highest plant density, combined with the most intensive N treatment. The smallest yield per hectare was observed in the treatment with the highest plant density, combined with no N treatment.

Sowing time is equally important. Early sowing can enhance germination and promote stronger initial growth due to increased soil moisture availability [Hadnagy 1981, Sreelatha et al. 1997, Prasad et al. 2002, McCormack 2004]. In contrast, late sowing often exposes plants to drought stress during critical growth phases. The later sowing date decreases the number of pods per plant, seed yield per plant, and yield per hectare [Bayrak et al. 2022]. Several studies have demonstrated the significant impact of sowing time and variety on pod number, seed number, seed yield per plant, seed number per pod, and thousand-seed weight. Postponing the sowing date decreases the crop components' yield and value [Jan et al. 2002, Mirzaienasab and Mojaddam 2014, Uddin et al. 2017].

While the effects of sowing time, plant density, and fertilisation have been studied, there remains limited information on how these factors interact under specific environmental and soil conditions, especially in Hungary.

This study evaluated the effects of sowing time, plant density, and fertiliser dosage on the yield and components in three dry bean varieties cultivated under field conditions in Eastern Hungary.

MATERIAL AND METHODS

The study was based on a 3-year series of experiments that we set up in Nyíregyháza (Hungary, GPS coordinates: 47.974961, 21.691528, located at approximately 100 meters above sea level) in 2015–2017. It involved three sowing dates, plant densities, fertiliser doses, and dry bean varieties, replicated four times in a randomised arrangement without irrigation. Each plot measured 10 m², and the sampling area within the plots was 0.25 m². In 2015, the experiment was conducted on a site with naturally lower humus content (0.84%), whereas in 2016–2017, it was relocated to a different area with more favourable soil conditions and a higher humus content (2.00%). In 2015 and 2016, the experiment was conducted on slightly acidic soils with (pH_{KCl}) values of 6.00 and 5.58, respectively, while in 2017, the trial was set up on neu-tral soil with a (pH_{KCl}) of 7.12. The soil type was classified as sand in 2015 and sandy loam in the following two years. Potassium supply was outstanding in 2015 and 2017 (AL-soluble K_2O: 247 and 328 mg kg^{-1}) and good in 2016 (211 mg kg^{-1}). Phosphorus availability was medium in 2015 (AL-soluble P_2O_5: 96 mg kg^{-1}) and good in 2016 and 2017, reaching 123 and 142 mg kg^{-1}, respectively. Nitrogen supply was rated as medium in 2015 (KCl-soluble NO_3^- + NO_2^-–N: 10 mg kg^{-1}) and good in the subsequent two years, with concentrations of 52 and 36 mg kg^{-1}, respectively. According to the WRB, the soil type is Arenosol and Aric [IUSS 2022].

The preceding crops were triticale (*Triticosecale* Wittm.) in 2014, buckwheat (*Fagopyrum esculentum* L.) in 2015, and oats (*Avena sativa* L.) in 2016. Fertiliser was applied before sowing, and soil was prepared using a combinator.

The sowing times: the most common in Hungary (between May 7–10), an earlier when the soil temperature increased permanently above 12 °C, and a later which was sowed until May 20 in all three years. In our experiment, the sowing dates were as follows: in 2015, April 24, May 8, and May 19; in 2016, April 25, May 9, and May 19; and 2017, May 3, May 11, and May 23. Typically, there was a 14-day interval between the early and standard sowing dates, except

in 2017, which had a shorter 8-day difference. The interval between the standard and late sowing dates usually ranged from 10 to 12 days. Sowing was conducted with a row spacing of 0.5 m and a depth of 3 to 5 cm.

The plant densities were set at 200,000, 300,000, and 400,000 per hectare. The fertiliser doses were as follows: control (0%), 100%, and 150%. The 100% dose is based on the recommendations of Antal [1983] and Velich [1994], which are 95 kg of N, 40 kg of P, and 80 kg of K for a yield of 1 ton per hectare.

Our research incorporated three distinct dry bean varieties. The Hópehely variety is characterised by its bushlike growth and large white seeds, making it suitable for salad production in regions with higher humidity and cooler climates. The Diana variety presents pintotype variegated beans and exhibits semideterminate growth. As for the Start variety, it features small white pearl seeds and follows a bushlike growth pattern.

The weather was hot and dry in 2015, especially during flowering. In the 2015 growing season, 120 mm of rain fell. The average maximum temperatures at flowering for all three varieties averaged 31°C in the third sowing season, compared to 26.5 °C in the first two seasons. It was unfavourable for fertilisation and the development of pods and seeds. In 2016–2017, the temperature and rainfall were more favourable for beans' development and crop for-mation. In 2016, 254 mm of precipitation fell during the growing season; in 2017, the amount was 239 mm. The average temperature at flowering in 2016 was 28 °C, whereas in 2017, the average maximum temperature was lower at 26 °C. During the first two sowing seasons, the average maximum temperature at flowering was 29.7 °C in 2016 and 27.6 °C in 2017, which was offset by the increased rainfall.

We applied a preemergence herbicide mixture of S-metolachlor and linuron, followed by hoeing to maintain weedfree plots. Plant protection was carried out using the following treatments: first, copper hydroxide, then mancozeb and lambda-cyhalothrin, and finally, copper oxychloride and acetamiprid.

Each year, the crop from the first sowing date was harvested in early August, the second in mid-August, and the third in late August. Samples were collected from the two central rows. Over three years, six plants were sampled per plot at 200,000 plants/hectare, eight plants at 300,000 plants/hectare, and 10 plants at 400,000 plants/hectare. We recorded the pod number per plant and developed seeds per pod by test plot (0.25 m^2). We weighed the developed seeds and calculated the thousand seed weight and yield per plant. The yield per hectare was calculated based on the sum of yields from individual plants within the sample plots. The analysis of variance was conducted using the SPSS software package. We opted for a straightforward statistical method, using one-way ANOVA for each variety to emphasise significant differences in the yield parameters we studied. During the variance analysis, the results shown in the tables were evaluated as averages of the other two agronomic factors. The Tukey-b method was used for the homogeneous data set, and the Games-Howell method was applied to the non-homogeneous data set at a 5% significance level. The crop element values follow a pattern similar to an exponential distribution, resulting in an increased standard deviation. The variance is explained by anomalies in crop production resulting from extreme weather conditions and variations in treatments.

Unfortunately, in the case of the Hópehely variety, the crop was lost due to waterlogging during the second sowing time in 2016, so we cannot provide data on that variety for that year.

RESULTS

Yield per hectare

The plant densities did not significantly affect yield per hectare for no variety. The significant effect of the fertiliser doses was verified only in the case of the Hópehely variety in 2016 when the treatment without fertiliser yielded more than 56% than fertilised treatments (Table 1). The effect of sowing times varied by variety. Diana and Hópehely varieties consistently had higher yields in the first sowing times. In 2015, the third sowing date yielded the lowest production levels across all three varieties analysed. In 2015, Diana's yield from the first sowing date was twelve times higher than that from the third sowing date. In 2016, it was three times higher; in 2017, it was 85% higher. In the case of Hópehely, the first sowing in 2015 produced 3.7 times more than the third sowing, and in 2017, this difference increased to five times. In 2015, the yield for the Start variety from the second sowing

Table 1. Effect of the sowing time, plant density and fertiliser dose on the yield (mean ±standard deviation, kg ha^{-1})

Value	Diana			Start			Hópehely		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Sowing times									
1	351 ±179a	1638 ±701a	2373 ±912a	343 ±195b	1187 ±538c	2724 ±851a	374 ±246a	1419 ±800a	3592 ±788a
2	144 ±197b	567 ±465b	1938 ±705a	1005 ±463a	1630 ±615b	2607 ±814a	151 ±104b	_	2884 ±858b
3	30 ±32c	556 ±403b	1282 ±499b	38 ±56c	2025 ±540a	1911 ±422b	101 ±99b	1188 ±520a	713 ±372c
Plant densities (thousand plants per hectare)									
200	196 ±225a	915 ±659a	2104 ±1034a	567 ±568a	1524 ±675a	2453 ±789a	223 ±241a	1152 ±724a	2728 ±1443a
300	180 ±224a	1051 ±875a	1827 ±781a	564 ±492a	1765 ±654a	2496 ±851a	169 ±133a	1400 ±686a	2577 ±1413a
400	146 ±153a	799 ±665a	1685 ±664a	382 ±423a	1579 ±635a	2337 ±790a	247 ±248a	1337 ±631a	2326 ±1285a
Fertiliser doses (%)									
0	231 ±208a	1072 ±674a	1816 ±918a	487 ±580a	1700 ±497a	2354 ±756a	248 ±160a	1717 ±711a	2365 ±1334a
100	127 ±167a	836 ±816a	1837 ±804a	513 ±479a	1463 ±606a	2446 ±741a	189 ±193a	1100 ±645b	2893 ±1241a
150	169 ±224a	853 ±716a	1956 ±837a	439 ±436a	1707 ±809a	2479 ±920a	202 ±255a	1079 ±465b	2422 ±1521a

Different letters indicate statistical difference ($p < 0.05$)

Table 2. Effect of the sowing time, plant density and fertiliser dose on the pod number per plant (mean ±standard deviation)

Value	Diana			Start			Hópehely		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Sowing times									
1	3.4 ±2.0a	5.5 ±3.5a	9.5 ±6.5a	7.6 ±6.2b	22.2 ±15.7a	22.8 ±11.9a	4.1 ±2.4b	9.8 ±5.7a	9.7 ±4.9a
2	3.8 ±2.6a	4.6 ±3.2b	9.4 ±6.0a	16.6 ±13.6a	15.3 ±9.8b	17.6 ±8.6b	6.4 ±3.8a	_	10.6 ±6.6a
3	1.7 ±1.0b	4.5 ±3.6b	6.7 ±4.3b	3.0 ±3.3c	18.7 ±10.7a	12.7 ±6.0c	3.4 ±2.3c	10.2 ±6.3a	6.7 ±3.3b
Plant densities (thousand plants per hectare)									
200	3.7 ±2.4a	6.4 ±4.1a	12.0 ±6.7a	12.9 ±13.4a	23.0 ±13.5a	20.6 ±11.1a	5.2 ±3.4a	12.2 ±7.8a	12.2 ±5.8a
300	3.7 ±2.4a	5.0 ±3.2b	8.5 ±5.7b	10.8 ±10.0b	17.3 ±9.9b	18.5 ±9.1a	4.4 ±3.2b	10.0 ±5.7b	9.3 ±5.6b
400	2.6 ±1.5b	4.1 ±2.7c	6.6 ±4.3c	8.8 ±8.9b	15.4 ±10.8b	14.1 ±8.1b	3.8 ±2.1c	8.9 ±4.9b	7.2 ±3.9c
Fertiliser doses (%)									
0	3.7 ±2.5a	5.0 ±3.2a	8.3 ±6.2a	9.8 ±10.4a	14.6 ±9.6b	16.9 ±10.1a	4.4 ±3.0a	8.8 ±5.1b	8.2 ±4.6b
100	3.1 ±1.9b	4.9 ±3.5a	8.2 ±5.6a	11.3 ±12.1a	18.8 ±11.9a	16.6 ±9.1a	4.6 ±3.2a	10.4 ±6.3a	10.4 ±6.2a
150	3.0 ±1.8b	5.2 ±3.7a	9.3 ±5.7a	11.0 ±9.9a	21.2 ±12.5a	18.3 ±10.0a	4.2 ±2.6a	11.8 ±6.8a	9.4 ±5.3a

Different letters indicate statistical difference ($p < 0.05$)

date was 26 times greater than from the third. In 2016, the first sowing date had the lowest yield, while the third date saw a 70% increase. In 2017, the second sowing date outperformed the third by 36%.

Pod number per plant

There were significant effects of the sowing times, plant densities, and fertiliser doses in cases of three varieties (Table 2). The effects of the fertiliser doses depended on the varieties.

In 2015, the Diana variety had 23% more pods per plant without fertiliser than with the highest dose. In 2016, the Start variety produced 45% more pods under the highest fertiliser dose, while Hópehely had a 34% increase compared to the unfertilised control. In 2017, Hópehely showed 27% more pods with 100% fertiliser than in the untreated control.

The pod number per plant decreased as plant densities increased at the three varieties. In the case of the Start variety, the number of pods per plant decreased by 67–68% when plant density increased from 200,000 to 400,000 plants per hectare. For the Hópehely variety, the reduction reached 73%, while in 2017, it was 59%. Diana's decline in pod number ranged between 55% and 70%, depending on the year.

The third sowing time has had the lowest number of pods per plant of the three varieties. For the Diana variety, in 2016, the first sowing date produced 22% more pods; in 2017, the first two sowing dates produced 42% more pods than the third sowing time. For the Start variety, the sowing date, which results in the highest number of pods per plant, varied yearly. Compared to the sowing date with the lowest number of pods, the increase was 1.5 to 5.5 times. At the Hópehely variety, the pod number was the highest in the second sowing time. It was approximately twice as high as the third sowing time, with increases of 60% in 2017 and 88% in 2015.

Thousand-seed weight

The sowing time significantly affected the thousand-seed weight of the three varieties (Table 3). The effect of plant density was not significant on the thousand-seed weight of the Diana variety. The effect of fertiliser doses was not significant on the thousand-seed weight of the Start variety. The most considerable thousand-seed weight was at different yearly sowing times at the Diana variety.

The small-seeded Start variety showed a higher thousand-seed weight in the third sowing time, increasing by 25% in 2016 and 4% in 2017. The large-seeded Hópehely variety had its highest thousand-seed weight at the first sowing, with 70% and 31% increases over two years. Thousand-seed weight consistently rose with earlier sowing dates.

In 2017, higher plant density led to a decrease in weight per thousand seeds for the Start variety. The highest weight was at 200,000 plant density, 3% higher than 400,000. Conversely, at the Hópehely variety, we observed different results in two years. In 2015, the thousand-seed weight at the 400,000-plant density was 7% higher than at the 200,000-plant density. However, in 2016, the 200,000-plant density had a significantly higher – by 5% thousand-seed weight than the value of the 400,000-plant density.

The effect of fertiliser doses depended on the varieties. Significant differences were observed in one year for both the Start and Hópehely varieties, while in the case of the Diana variety, significant differences were confirmed in two years. In 2016, for the Hópehely variety, the thousand-seed weights in the fertilised treatments were 5% and 6% higher than in the unfertilised control. Still, the value of the treatment without fertiliser did not differ significantly from the value of the 150% fertiliser doses. In the case of the Start variety, the thousand-seed weight in the fertilised treatments was significantly higher, by 5%, than that of the untreated control in 2016. For the Diana variety, fertilised treatments consistently produced higher values – by 6% and 4% in 2016 and 2017, respectively, with the 150% dose producing a signifi-cantly greater thousand-seed weight than the control under all conditions.

Well-developed seed number per pod

In 2016, the well-developed seed number per pod of the first sowing time was the largest of the three varieties (Table 4). For Diana, the increase was 28%; for Start, 42%; and for Hópehely, 45%. 2015 was a varied year; for Diana and Hópehely, the highest values were recorded at the third sowing time, 36% and 30% higher, respectively, compared to the lowest at the second sowing date. The Start variety had the most significant well-developed seed number at the second sowing time, 58% greater than the first.

Table 3. Effect of the sowing time, plant density and fertiliser dose on the thousand-seed weight (mean ±standard deviation, g)

Value	Diana			Start			Hópehely		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Sowing times									
1	274 ±39b	264 ±41b	265 ±41a	187 ±35a	163 ±23c	161 ±14b	363 ±94a	306 ±46b	374 ±31a
2	288 ±43a	266 ±51b	255 ±40b	161 ±29b	180 ±21b	162 ±15b	254 ±38b	–	364 ±36b
3	260 ±50b	296 ±56a	250 ±33b	180 ±44a	203 ±24a	168 ±14a	214 ±46c	392 ±52a	285 ±34c
Plant densities (thousand plants per hectare)									
200	275 ±43a	278 ±48a	261 ±39a	173 ±34a	186 ±27a	167 ±14a	293 ±97b	362 ±71a	349 ±50a
300	280 ±41a	269 ±51a	256 ±37a	179 ±38a	187 ±28a	164 ±15ab	296 ±96ab	346 ±64ab	349 ±51a
400	274 ±42a	272 ±49a	254 ±39a	175 ±36a	187 ±24a	162 ±15b	314 ±104a	344 ±62b	346 ±50a
Fertiliser doses (%)									
0	276 ±41a	265 ±49b	253 ±39b	175 ±35a	182 ±26b	166 ±16a	277 ±89a	338 ±65b	343 ±50a
100	278 ±43a	275 ±49a	255 ±35ab	175 ±36a	191 ±28a	163 ±14a	304 ±98a	360 ±69a	350 ±46a
150	275 ±43a	280 ±51a	262 ±40a	178 ±38a	191 ±25a	163 ±14a	335 ±104a	354 ±60ab	351 ±54a

Different letters indicate statistical difference ($p < 0.05$); sowing times: 1 – early, 2 – standard, 3 – late

Table 4. Effect of the sowing time, plant density and fertiliser dose on the well-developed seed number per pod (mean ±standard deviation)

Value	Diana			Start			Hópehely		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Sowing times									
1	1.8 ±0.9a	2.3 ±1.1a	3.1 ±1.0a	1.2 ±0.5c	2.7 ±0.7a	3.3 ±0.6a	1.1 ±0.6b	1.6 ±0.7a	2.7 ±0.7a
2	1.4 ±0.8b	1.8 ±1.0b	2.7 ±1.0b	1.9 ±0.7a	2.1 ±0.6b	3.1 ±0.7b	1.0 ±0.6c	–	2.3 ±0.7b
3	1.9 ±1.0a	1.8 ±1.0b	2.5 ±1.0c	1.4 ±0.8b	1.9 ±0.5c	2.8 ±0.5c	1.3 ±0.8a	1.1 ±0.6b	1.9 ±0.6c
Plant densities (thousand plants per hectare)									
200	1.7 ±0.8a	2.0 ±1.0a	2.9 ±1.0a	1.5 ±0.7a	2.2 ±0.6a	3.1 ±0.6a	1.1 ±0.7b	1.4 ±0.7a	2.3 ±0.9a
300	1.7 ±0.9a	2.1 ±1.0a	2.8 ±1.0ab	1.5 ±0.8a	2.2 ±0.7a	31 ±0.6a	1.1 ±0.6b	1.3 ±0.8a	2.2 ±0.9a
400	1.8 ±0.9a	2.1 ±1.1a	2.7 ±1.0b	1.5 ±0.7a	2.0 ±0.6b	2.9 ±0.7b	1.2 ±0.7a	1.3 ±0.7a	2.1 ±0.9a
Fertiliser doses (%)									
0	1.6 ±0.8b	2.2 ±1.1a	2.9 ±1.1a	1.5 ±0.8a	2.1 ±0.7a	3.0 ±0.6a	1.2 ±0.7a	1.5 ±0.8a	2.2 ±0.9ab
100	1.7 ±0.9ab	2.1 ±1.1ab	2.7 ±1.1a	1.5 ±0.7a	2.2 ±0.6a	3.1 ±0.7a	1.2 ±0.7a	1.3 ±0.7b	2.3 ±0.8a
150	1.9 ±1.0a	1.9 ±1.0b	2.8 ±0.9a	1.5 ±0.7a	2.2 ±0.7a	3.0 ±0,7a	1.1 ±0.6a	1.1 ±0.6b	2.1 ±0.9b

Different letters indicate statistical difference ($p < 0.05$)

Plant densities had differing effects on Diana, Hópehely, and Start varieties. For Diana, in 2017, the number of well-developed seeds per pod was 7% higher at the 200,000 plant densi-ty than at the 400,000 plant density. In the case of Hópehely, under the highest density (400,000 plants per hectare), 9% more well-developed seeds were formed in 2015 compared to the lower densities. The Start variety had the lowest well-developed seed number at 400 thousand plant density during 2016–2017.

The fertiliser doses had varied effects on analysed plant varieties. The Start variety showed no significant effect. In the case of Diana, the 150% dose in 2015 resulted in an 18% increase in the number of well-developed seeds compared to the unfertilised control, while in 2016, the no-fertiliser treatment produced 16% more well-developed seeds than the 150% dose. For the Hópehely variety, the highest value in 2016 was recorded in the unfertilised treatment, which yielded 36% more well-developed seeds than the 150% dose. In contrast, in 2017, the 100% dose outperformed the 150% treatment by 10%, showing a significantly higher result.

Yield per plant

The significant effects of sowing times and plant densities were verified yearly (Table 5). In 2016, the Diana variety yielded 60% more per plant at the first sowing date, and the Start variety 72% more per plant than at the second sowing date, with the lowest yield. In 2017, the Diana variety yielded 89% more per plant at the first sowing date, and the Start variety twice as much as the third sowing date, with the lowest yield. The Hópehely variety yielded 89% more at the first sowing date in 2015 and four times more at the third sowing date in 2017. Regarding the three varieties, the yield per plant was the significant least in the third sowing time in 2015 and 2017.

The values decreased with an increase in plant densities. The lowest values were in 400,000 plant densities. The 200,000-plant density resulted in significantly higher yield per plant: 38–200% more for Diana, 53–65% more for Start, and 52–81% more for Hópehely. In 2016, there were no significant differences in values between 300,000 and 400,000 plant densities for the Hópehely and Start varieties, similar to the situation with the Start variety in 2015.

The fertiliser doses did not significantly affect the Diana variety. For other varieties, there was only a significant effect in one year. The Start variety had a significantly higher yield per plant with fertiliser treatments – by 38% and 63% – in 2016. In 2017, the 100% fertiliser dose treatment had the highest value, producing 33% more than the unfertilised control for the Hópehely variety, while the values without fertiliser and with a 150% dose did not differ significantly.

DISCUSSION

Our research aimed to identify the best technology for growing three common dry bean varieties. The common bean is sensitive to weather conditions, especially temperature and rainfall, which significantly impact the reproductive development stages. Similar observations were made in 2015, as reported by Ovacikli and Tolay [2020] and Kádár [2013]. During the flowering and fruiting period of the third sowing season, prolonged heat and a lack of rainfall occurred, leading to an atmospheric drought that caused a significant yield reduction.

Among the parameters studied, sowing time had the most significant impact on variations, followed by plant density. Fertilisation only led to a significant difference in one instance: the Hópehely variety of white salad beans yielded more without fertiliser than with it. This aligns with Kádár's [2013] finding that fertilisation can adversely affect unfavourable weather conditions. This variety prefers a cooler climate and yielded more during the initial sowing period, which corresponds to the findings of Jan et al. [2002], Mirzaienasab and Mojaddam [2014], and Uddin et al. [2017]. Late sowing often exposes plants to drought stress during critical growth phases [Bayrak et al. 2022]. Diana pinto beans yield more with early sowing, as their mottled seed coat offers better resistance to infections even in cooler soils after sowing [McCormack 2004]. The Start variety does better with later sowings due to its short growing period and small white seeds [McCormack 2004]. According to Hadnagy [1981], short-growing varieties in Hungary can reach maturity even if sown as late as May 20.

In the case of the Start and Hópehely varieties, fertilisation resulted in more pods per plant, consistent with the literature [Begum et al. 2003, Shubhashree

Table 5. Effect of the sowing time, plant density and fertiliser dose on the yield per plant (mean ±standard deviation, g)

Value	Diana			Start			Hópehely		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Sowing times									
1	1.8 ±1.4a	3.5 ±3.1a	8.5 ±7.7a	1.8 ±1.9b	10.5 ±8.9a	12.5 ±7.6a	1.7 ±1.4a	5.0 ±3.8a	10.0 ±5.8a
2	1.6 ±1.6a	2.2 ±2.1b	7.1 ±6.4b	6.0 ±6.4a	6.1 ±4.8c	9.1 ±5.5b	1.5 ±1.4a	–	9.4 ±7.0a
3	0.8 ±0.8b	2.5 ±2.6b	4.5 ±3.9c	0.7 ±0.7c	7.4 ±5.1b	6.0 ±3.3c	0.9 ±0.8b	4.5 ±4.2a	2.4 ±2.0b
Plant densities (thousand plants per hectare)									
200	1.8 ±1.6a	3.6 ±3.2a	9.9 ±8.1a	4.3 ±6.3a	9.5 ±7.1a	11.2 ±7.3a	1.5 ±1.4a	6.1 ±4.9a	10.7 ±7.6a
300	1.8 ±1.6a	2.9 ±2.9b	6.7 ±6.2b	3.4 ±4.5ab	7.3 ±5.4b	9.6 ±5.7b	1.3 ±1.1a	4.7 ±4.0b	8.0 ±6.6b
400	1.3 ±1.0b	2.4 ±2.2c	4.9 ±4.4c	2.6 ±3.5b	6.2 ±5.3b	6.9 ±4.7c	1.5 ±1.4a	4.0 ±3.2b	5.9 ±4.6c
Fertiliser doses (%)									
0	1.7 ±1.6a	2.9 ±2.5a	6.6 ±7.1a	3.3 ±4.9a	5.7 ±4.7b	8.7 ±6.2a	1.4 ±1.3a	4.5 ±3.8a	6.9 ±6.0b
100	1.5 ±1.4a	2.9 ±3.0a	6.3 ±6.1a	3.5 ±5.3a	7.9 ±6.3a	8.7 ±6.0a	1.5 ±1.4a	4.8 ±4.1a	9.2 ±7.1a
150	1.7 ±1.3a	3.0 ±3.1a	7.3 ±5.9a	3.4 ±4.1a	9.3 ±6.6a	9.3 ±6.1a	1.5 ±1.4a	5.0 ±4.3a	7.7 ±6.1b

Different letters indicate statistical difference ($p < 0.05$)

et al. 2011, Faria and Fageria 2014]. For the Diana variety, more pods were observed in the unfertilised treatment, which was confirmed in one year of the study. Our findings on plant densities and sowing time align with the literature [Moniruzzaman et al. 2009, Mekonnen et al. 2012, Tuarira and Moses 2014, Seif et al. 2016, Soratto et al. 2017]. The value increases at lower plant densities and decreases with increasing density. Similarly, the value was smallest when sowing was done at the latest time.

The timing of sowing significantly affected the thousand-seed weight for all three varieties. Early sowing resulted in the highest weight for the Hópehely variety, while the Start variety had lower weights during early sowing. The Diana variety showed varied effects of sowing time on thousand-seed weight each year. Our observations align with Mekonnen et al. [2012], Ahmed et al. [2016], and Seif et al. [2016], indicating that thousand-seed weight decreases with higher plant density. The effect of fertiliser doses on thousand-seed weight depended on the variety.

The value of the number of well-developed seeds per pod was significantly highest in the first sowing time regarding all three varieties in 2016–2017, as also reported by Mirzaienasab and Mojaddam [2014] and Uddin et al. [2017]. As the literature [Jan et al. 2002, Bakure et al. 2023] appropriately suggests, the number of well-developed seeds decreased with increased stocking density in the Start and Diana varieties. The Start variety showed no significant effect from fertiliser doses, while the effect varied among the other varieties.

The study confirmed the significant impact of sowing time and plant density on yield per plant over three years. The yield per plant decreased as plant densities increased, as also observed in the study by Shiv Kumar and Mishra [2002]. The fertilisation had minimal effect, possibly due to lack of irrigation. To maximise yield, it is crucial to determine the best sowing time, plant density, and fertiliser dose for the specific crop variety, which are influenced by factors like growth type and seed colour.

SUMMARY

Our three-year field study evaluated the effects of sowing time, plant density, and fertiliser dose on the yield and yield components of three dry bean varieties under Eastern Hungarian conditions. The results showed that the response of each variety differed depending on the examined agronomic factors.

The Diana variety showed the best performance when sown early, resulting in high pod numbers, well-developed seeds per pod, and overall yield. Increasing plant density negatively affected yield components for all varieties. The impact of fertiliser application varied by year; in the case of Diana, it did not consistently lead to increased yield. However, for the Start and Hópehely varieties, specific fertiliser treatments did result in improved performance.

The Start variety showed flexibility in sowing time, with satisfactory yields on standard and late sowing dates. The large-seeded Hópehely variety responded best to early sowing and moderate plant density.

These results demonstrate the necessity for tailored farming strategies for different dry bean varieties. Future research must focus on understanding how these varieties respond to environmental conditions and determining the optimal input levels for sustainable growth.

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EFFICACY OF SEVERAL INSECTICIDES AND PLANT EXTRACTS AGAINST *Ostrinia nubilalis* HÜBNER IN SWEET PEPPER

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ABSTRACT

Pepper (*Capsicum annuum* L.) is a globally important crop, often affected by the European corn borer (*Ostrinia nubilalis* Hübner, ECB), a significant pest causing substantial yield losses. Chemical insecticides, such as chlorantraniliprole and indoxacarb, are commonly used to control this pest; however, their environmental and health risks and potential for resistance development highlight the need for alternative pest management strategies. Biological control methods offer promising alternatives, including natural enemies and plant-derived compounds. This study evaluated the efficacy of neem oil, garlic extract, and chili pepper extracts against ECB larvae on pepper plants, comparing them to the chemical insecticides indoxacarb and chlorantraniliprole. Results from a two-year field experiment indicated that chlorantraniliprole exhibited the highest efficacy in reducing pepper fruit damage and larval survival, with the lowest damage severity and the lowest number of larvae per fruit. In the first year, neem oil and garlic extract showed promising results, effectively reducing fruit damage and performing comparably to indoxacarb. However, in the second year, only chlorantraniliprole showed consistent efficacy, likely due to unfavorable weather conditions that reduced the persistence of other treatments. Compared to the control, plant extracts showed higher efficacy in the first year of the trial, while indoxacarb was equally effective as the plant extracts. These findings suggest that plant-derived products like neem oil and garlic extract may be viable alternatives to chemical insecticides; however, further investigation is needed to optimize their application and efficacy in pest control.

Keywords: *Capsicum annuum*, garlic extract, chili extract, neem oil, European corn borer control, chlorantraniliprole

INTRODUCTION

Pepper (*Capsicum annuum* L.) is one of the most important vegetables and spice crops globally. The world's total cultivation area under pepper was above 2 million hectares in 2023 [FAOSTAT 2024]. Pepper is also one of the major vegetable species in Serbia, and it was grown at 9,915 ha in 2023 [FAOSTAT 2024]. Several major pests affect pepper crops, with the European corn borer (*Ostrinia nubilalis* Hübner, ECB) (Lepidoptera: Crambidae) causing the most substantial damage. Although the primary host plant of ECB is maize [Schmidt-Jeffris and Nault 2017], in recent years, it has gained increasing importance as a pest of pepper [Sekulić et al. 2003]. The main damage is caused by the caterpillars, which tunnel into the stem and fruit, feeding on the pericarp and seeds, weakening the plant and directly reducing yield. Infested pepper fruits eventually rot and drop off; late-infested fruit may exhibit premature reddening [Mason et al. 1996]. The economic losses can reach 50% or more [Sekulić et al. 2003]. The larvae are difficult to detect, and infestations are typically noticed only after significant damage. The larvae

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move among fruits, spreading pathogens such as Pectobacterium carotovorum (Jones) Waldee, and rotten fruits are generally symptoms of ECB infestation [CABI 2021]. Given this species' highly detrimental effect on pepper production, chemical treatments are often used to control the pest. However, insecticide residues in pepper fruits pose a serious threat to both consumers and the environment. Chlorantraniliprole and indoxacarb are the most commonly used chemical insecticides to control ECB infestations.

Chlorantraniliprole is an insecticide belonging to the anthranilic diamide class, first registered for use in 2008. It controls Lepidopteran, Coleopteran, and certain Dipteran pests [Bentley et al. 2010]. Indoxacarb is a highly toxic, broad-spectrum oxadiazine insecticide used against several Lepidopteran pests and certain Hemiptera and Coleoptera [Wing et al. 2000].

Likewise, the excessive use of insecticides may lead to the development of resistance to chemical compounds. Chlorantraniliprole-resistant strains of *Spodoptera littoralis* Boisduval showed obvious cross-resistance to indoxacarb [Moustafa et al. 2024].

On the other hand, biological control-based solutions, as the application of natural enemies, like parasitic wasps (*Trichogramma* sp.) and biological insecticides based on *Bacillus thuringiensis* subsp. *kurstaki*, are promising alternative control measures still underused, primarily due to the challenges associated with their application. A promising alternative is the use of plant-derived oils and extracts. These compounds can potentially reach pest control products' efficacy and remain safe for consumers and the environment [Souto et al. 2021]. Additionally, botanical extracts provide innovative and various modes of action that reduce the probability of developing resistance in pest populations [Isman 2008].

Extracts from several plant species, such as garlic, chili peppers, and neem, have shown strong insecticidal properties [Ngegba et al. 2022]. Garlic (*Allium sativum* L.) has insecticidal, repellent, anti-feeding, and broad-spectrum anti-microbial properties [Mamduh et al. 2017]. It promotes self-defense mechanisms in plants against fungal and bacterial infections, acts as a biostimulant or inducer [Hayat et al. 2022], and could be used as a coating biofungicide before sowing for the disinfection of wheat seeds [Perelló et al. 2013]. Garlic and its extracts have shown high efficacy in controlling insect pests from several orders, such as Coleoptera [Lu and Liu 2003, Mamduh and Movahedi Fazel 2010, Beltagy and Omar 2016, Golubkina et al. 2022], Lepidoptera [Lu and Liu, 2003, Perez-Mendoza and Aguilera-Penã 2004, Oparaeke et al. 2007], Heteroptera [Jaastad et al. 2007, Golubkina et al. 2022], and Diptera [Prowse et al. 2006, Cao et al. 2012]. Capsaicin is the active ingredient in chili peppers. It is used as a bird and insect repellent. Capsaicin has also demonstrated insecticidal activity and can control a wide range of insect pests, but at low levels of insect infestation [Li et al. 2019]. Its main target pests are aphids, loopers, armyworms, spider mites, thrips, leaf miners, and whiteflies [Antonious et al. 2006, Tomita and Endo 2007, Cuadrado et al. 2019]. Capsaicin has also shown anti-microbial activity [Vuerich et al. 2023]. Neem oil is an organic biopesticide extracted from the fruits of the neem tree, *Azadirachta indica* A. Juss. The primary active ingredient of most neem-derived products is azadirachtin. This steroid-like tetranortriterpenoid exhibits a wide range of bioactivity to hundreds of phytophagous insect species from different orders [Shannag et al. 2015], among which it has shown antifeeding effects and increased larval mortality in Lepidoptera [Mancebo et al. 2002]. This study aimed to assess the efficacy of neem oil, garlic, and chili pepper extracts against ECB larvae on pepper plants and compare it with two widely used active ingredients in commercial pesticides, indoxacarb, and chlorantraniliprole, in order to find out if botanicals can provide adequate alternative for chemical insecticides.

MATERIAL AND METHODS

Experimental design

The two-year trial was carried out in 2018 and 2019 on an experimental field of the Institute of Field and Vegetable Crops (Rimski Šančevi, Novi Sad, Serbia). The pepper variety Amfora (NS Seme) was sown on the 4th of April 2018 and 28th of March 2019 in a plastic greenhouse without heating. Amfora is a sweet pepper variety belonging to the "kapia" type, the most preferred pepper fruit type in Serbia [Danojević et al. 2021]. This variety was chosen because it is

extensively grown and because the damage of the ECB is more severe in sweet pepper varieties with larger fruits [Sekulić et al. 2003].

A herbicide based on diquat 200 g L^{-1} (3 ml in 1 L of water) was used before the emergence, while during transplants, growing fungicide based on a.i. propamocarb-fosetylate 840 g L^{-1}, in a dose of 3 mL in 2 L of water (5 L solution per 1 m^2) was applied, and 2% solution of water-soluble fertilizer NPK 15:30:15. Plants were transplanted into an open field on the 31st of May 2018 and the 14th of June 2019.

The size of the basic experimental plot was 5×1.4 m. The planting distance was 70 cm between rows and 25 cm within the row. The experiment was established in a randomized block design with 3 replications with 40 plants per replicate. Fertilization with ammonium nitrate was applied at a rate of 200 kg ha^{-1} on the 26th of June 2018 and the 5th of July 2019. The plants were irrigated with sprinklers. No other pesticides were applied after transplanting.

Laboratory egg masses rearing

In order to increase pest pressure, one cluster of ECB eggs (30 eggs on average) per plant was placed onto the inner leaves on the upper third of 10 pepper plants (per replicate) on the 24th of July 2018 and 29th of July 2019. The egg masses were obtained in laboratory conditions from wild-collected specimens. The moths were collected in light traps, and each morning, they were placed in rearing cages. The rearing cages comprised a wooden 50×50×50 cm frame covered with a mesh screen. A special opening for introducing fresh specimens and extracting dead ones was positioned on the side of the cage. The moths were kept at 25 ±2 °C, relative humidity: 45 ±5%, and photoperiod 14:10 (light:dark) and fed daily with a sugary syrup. A 50×50 cm filter paper was placed at the top of the cage on which the females laid eggs. The eggs were collected daily by cutting the paper around the egg masses, then stored at 25 °C for several days to check the eggs' quality before being placed on the pepper plants. Eggs were placed on ten healthy pepper plants (without symptoms of any diseases), one egg mass per plant, and attached with an entomological needle to the leaf. Inoculated plants were marked. In 2019, the egg clusters that dried out before hatching were replaced by fresh ones on the 4th and 5th of August. At the inoculation period, the first fruit on pepper plants has reached typical size and form (stage 701 according to the BBCH scale).

Plant extract preparation

Plant extracts were prepared as an infusion using an extraction technique reported by Azwanida [2015] and slightly modified. Garlic cloves of Bosut (autumn variety) were measured (10 g), crushed, and boiled in 500 mL water for 15 minutes. After boiling, the extract was cooled for 24 h, filtered, and diluted up to 1 L of water. Water garlic extract was used because the concentration of alicin, one of the main bioagents, is ten times higher than in ethanol extract [Bajac et al. 2018]. The same procedure was used for the preparation extract from physiologically mature fruits of the red Habanero variety of *Capsicum chinense* Jacq., except that 10 g of air-dried fruits cut in pieces were used. Commercial neem oil (concentration 100%) produced by Shree Baidyanath, India, was used for neem plant extract. For the treatment of pepper plants, the concentration of neem plant extract was 1% (10 mL of the neem oil was diluted in 1 L of water). Subsequently, after preparation, extracts were used for plant treatment.

Treatment application

The application of pesticides and plant extracts was done once per year – on the 30th of July 2018 and the 6th of August 2019, during calm weather (without wind). Three biological and two chemical insecticides (Table 1) were applied using a SOLO 417-Li backpack sprayer with a pressure of 2 bar. Untreated plants were used as a control. The amount of solution per replication (plot) was 280 mL.

Assessments

The pepper fruits from the inoculated and control plants were harvested on the 24th of August 2018 and the 4th of September 2019, at BBCH scale 805. The fruits were dissected, and each fruit's number of larvae and damage severity were noted. Five fruits per plant were evaluated according to the damage rating scale (Fig. 1). This scale was purposely designed for ECB damage evaluation on pepper. The values on the scale range from 0 to 5, meaning the rating 0 is given to pepper fruits with no sign of damage. Rating 1 (minimal

Table 1. Insecticides and plant extracts used in the experiment

Insecticide active ingredient	Rate per hectare (L ha^{-1})
chlorantraniliprole 200 g L^{-1}	0.1
indoxacarb 150 g L^{-1}	0.25
Plant extract	**Amount of plant extract per 1 L of water**
neem oil	10 mL
aqueous garlic extract	10 g
aqueous red habanero extract (chili)	10 g

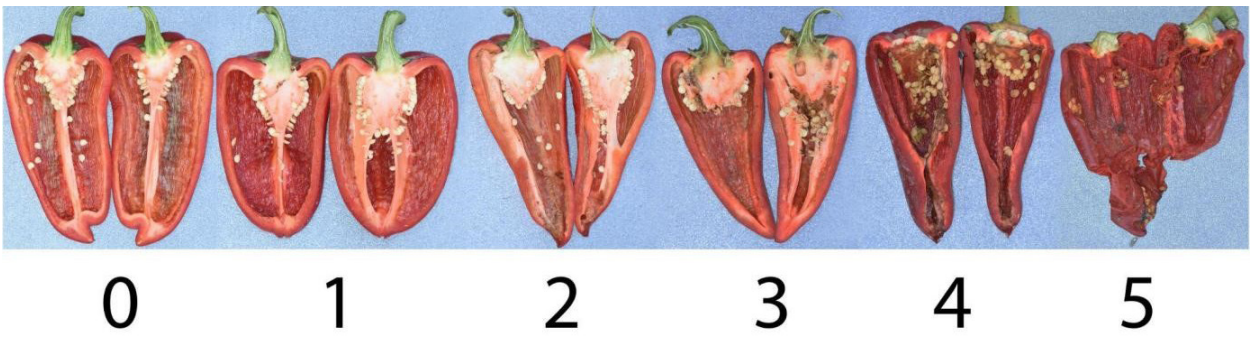

Fig. 1. Damage severity caused by *Ostrinia nubilalis* on pepper fruit (rating scale 0–5): 0 – no damage, 1 – minimal damage, 2 – visible damage, 3 – considerable damage, 4 – severe damage, 5 – destroyed fruit

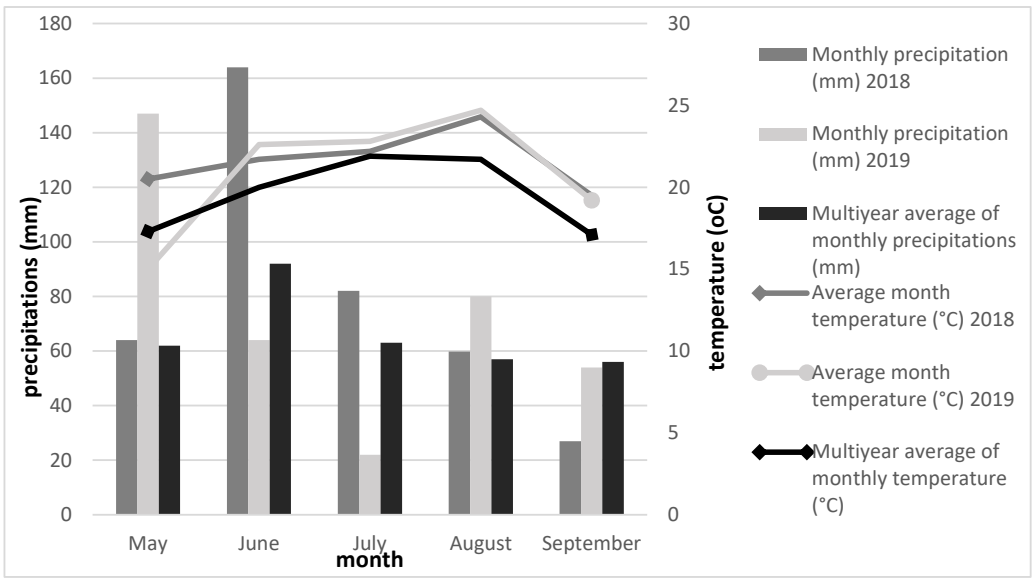

Fig. 2. Meteorological data for 2018 and 2019, compared with the 30-year climatic average (1989–2019) [Republic Hydrometeorological Service of Serbia]

Table 2. Analysis of variance (ANOVA) for the effects of active ingredient and year on damage severity and number of larvae in pepper fruits

Effect	df	Damage severity			Number of larvae		
		MS	F	*p*	MS	F	*p*
Intercept	1	1045.207	518.064	0.000	51.615	340.163	0.000
Active ingredient	6	33.308	16.510	0.000	2.294	15.120	0.000
Year	1	0.597	0.296	0.586	0.036	0.237	0.627
Active ingredient * year	4	2.147	1.064	0.373	0.071	0.470	0.758
Error	1755	2.018	–	–	0.151	–	–

df – degree of freedom, MS – mean square, F – value, *p* – probability

damage) is given to fruits with barely visible damage, rating 2 (clearly visible damage) is given to fruits showing visible but limited damage without the presence of frass, rating 3 (considerable damage) is given to fruits with a large amount of damage and visible frass, while rating 4 (severe damage) is given to fruits that not only exhibit extensive damage and frass but also show signs of decomposition visible externally, without the need for dissection. The highest rating (5) is given to fruits with such an extent of damage, already in an advanced stage of decomposition, and can be classified as destroyed. The commonly used tunnel length assessment method [Butron et al. 2014] for stem-boring insects in maize could not be applied to pepper fruits due to the complex tunneling patterns created by ECB larvae as they feed on them. Therefore, the authors devised an alternative method that has demonstrated ease of use and produced reliable results.

Statistical analysis

A two-factorial experimental design was applied, with year and active ingredient as fixed factors. The experiment was conducted over 2 years to assess the impact of various insecticides and plant extracts on damage severity and the number of larvae in pepper fruits. The data were analyzed using ANOVA with type I sum of squares and sigma-restricted parameterization due to many zero values in the dataset. The model included an intercept term, as it was found to be necessary for accurate estimation. The results were presented graphically for each year separately to enhance clarity and facilitate comparison. Post-hoc comparisons were conducted using the Bonferroni test at a significance level 0.05. Statistica software version 13.2 (Dell Inc., USA) was applied for these analyses.

Meteorological conditions

Meteorological conditions during the two experimental years varied considerably compared to the 30-year climatic average (1989–2019), as officially reported by the Republic Hydrometeorological Service of Serbia. In the first experimental year, 2018, a large amount of precipitation was recorded in June, and the temperatures were above average. During the experimental setup in August, the average temperatures were above the multiyear average for that month (Fig. 2). In the second experimental year, 2019, a significant amount of rainfall was recorded in May, while there was a notable lack of rainfall in July. The average temperature in August was almost 3 °C higher than the multiyear average.

RESULTS

The ANOVA results indicate that the active ingredient had a highly significant effect on both damage severity and number of larvae ($p < 0.001$), suggesting that the treatments differed significantly in their effectiveness. However, year and the interaction between active ingredient and year did not show statistically significant effects for either parameter ($p > 0.05$), implying that the treatments produced consistent results across both years and that seasonal variation (likely due to meteorological conditions) did not significantly influence the outcomes (Table 2). This suggests that the active ingredients tested in this study were effective regardless of the year or the environmental conditions.

Although the interaction between active ingredient and year was not statistically significant, the results were presented separately for each year to allow a more straightforward interpretation of treatment per-

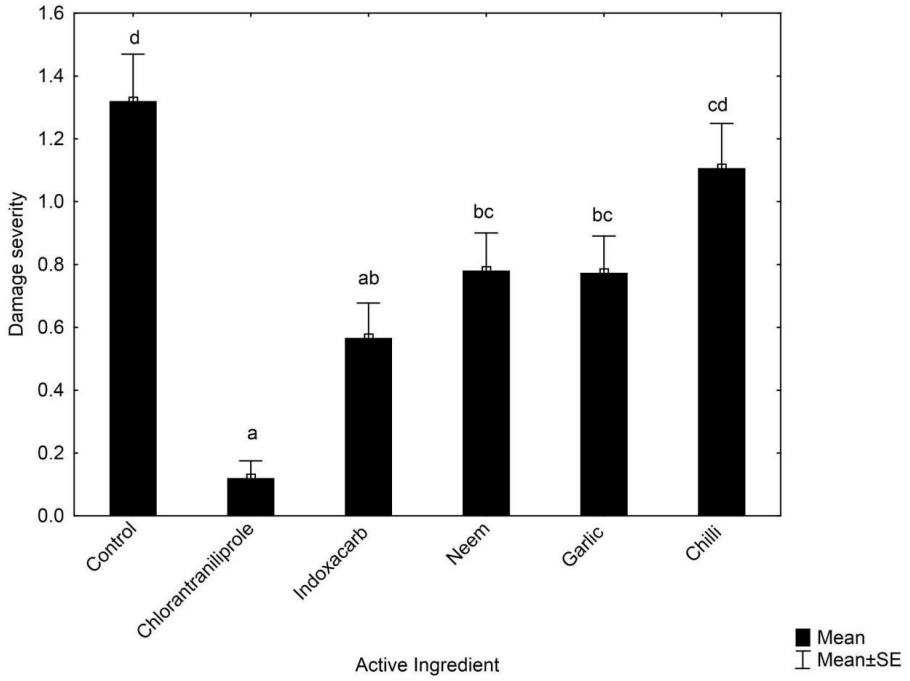

Fig. 3. Effects of different active ingredients on pepper fruit damage severity caused by *O. nubilalis* in 2018. Different letters indicate a significant difference, as determined by the Bonferroni test ($p < 0.05$)

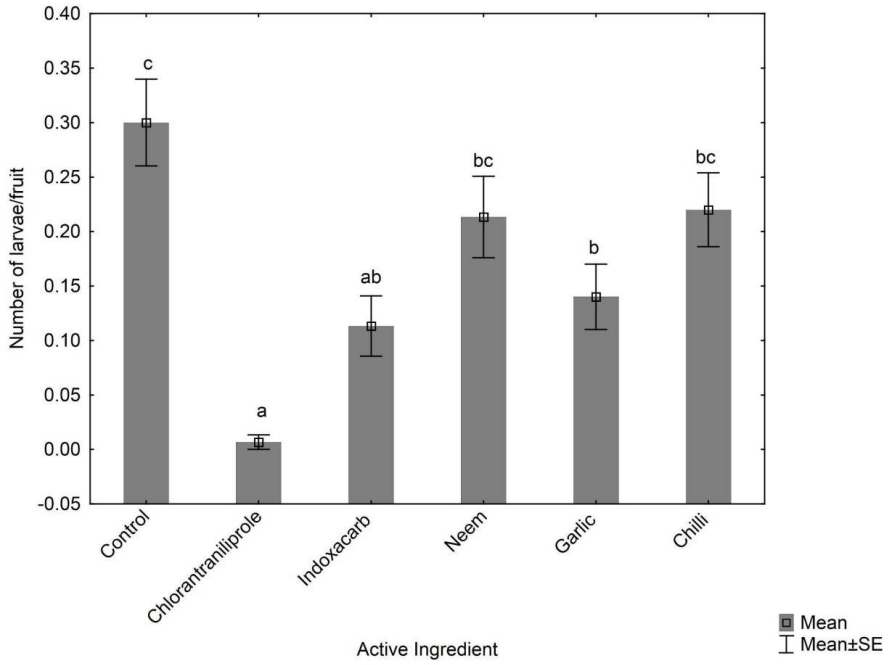

Fig. 4. Effects of different active ingredients on the number of larvae in pepper fruit in 2018. Different letters indicate a significant difference, as determined by the Bonferroni test ($p < 0.05$)

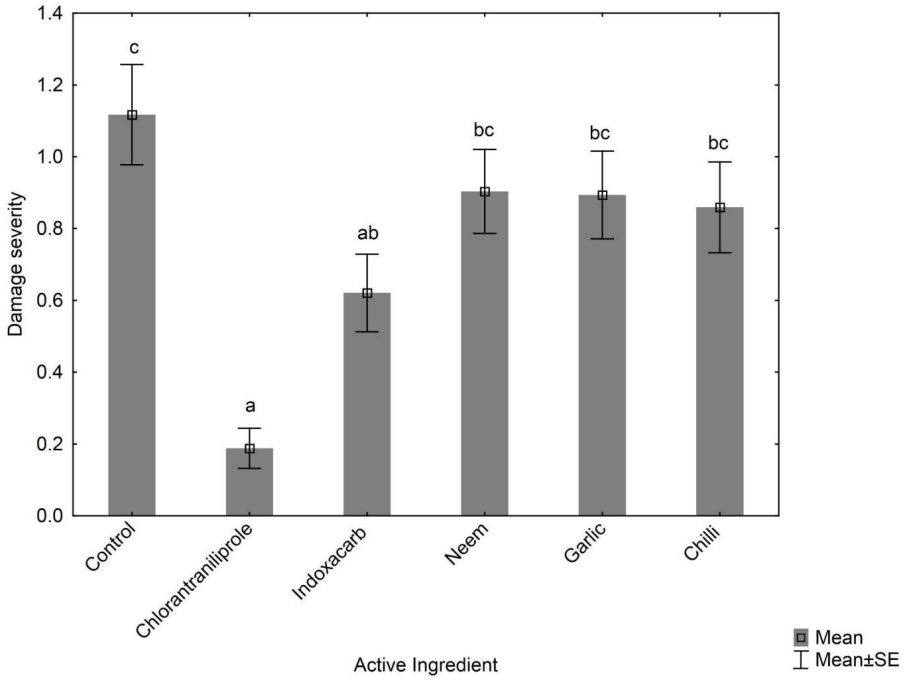

Fig. 5. Effects of different active ingredients on pepper fruit damage severity in 2019. Different letters indicate a significant difference, as determined by the Bonferroni test ($p < 0.05$)

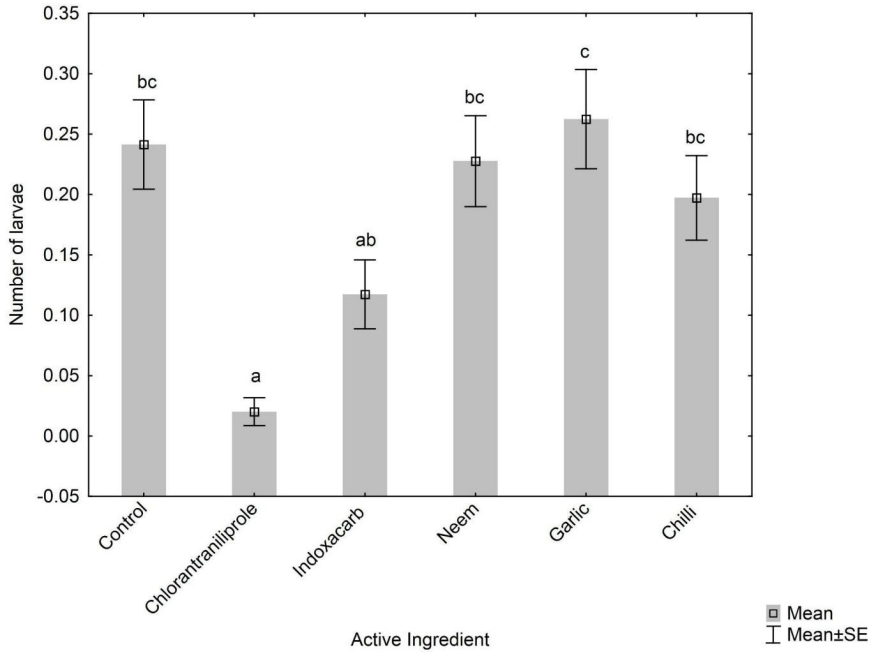

Fig. 6. Effects of different active ingredients on the number of larvae in pepper fruit in 2019. Different letters indicate a significant difference, as determined by the Bonferroni test ($p < 0.05$)

formance under differing environmental conditions. This approach provides insight into potential year-specific trends or anomalies, such as the observed reduction in efficacy during the second year, likely due to unusual weather patterns.

Results from 2018

The results for the first year of the trial showed that the lowest damage severity caused by ECB larvae was observed on pepper fruits treated with chlorantraniliprole (0.12), while the highest was in the control treatment – 1.32 (Fig. 3). All treatments, except chili treatment, showed statistically significant differences compared to the control; however, there was no significant difference between chlorantraniliprole and indoxacarb. Furthermore, the chemical insecticide based on indoxacarb did not show a significant difference in fruit damage severity compared to neem oil and garlic aqueous extract. Analyzing the effects of used plant solutions (neem oil, garlic, and chili aqueous extracts), the highest damage severity in 2018 was noted on pepper fruits treated with chili extract – 1.11 (Fig. 3).

In the first experimental year, pepper fruits treated with insecticide based on chlorantraniliprole were free of ECB larvae – 0.0, while the highest number was observed in the control plot – 0.3 (Fig. 4). Similar to the damage severity stage (level), there was no significant difference between chlorantraniliprole and indoxacarb in the number of larvae per fruit. Moreover, differences between indoxacarb, neem, garlic, and chili were not significant, but the difference between garlic – 0.14, and indoxacarb – 0.11, was very low (Fig. 4).

Results from 2019

In the second year of the experiment (2019), similar to the previous year, the lowest damage severity on pepper fruits was observed in the chlorantraniliprole treatment, while the control treatment showed the highest damage (Fig. 5). The treatment with indoxacarb (0.62) showed greater damage on pepper fruits than chlorantraniliprole (0.19). Extracts made from plants (neem, garlic, chili) showed similar effects on damage severity in 2019, with no significant differences compared to the control.

The lowest number of larvae was observed in the treatment with chlorantraniliprole, the only treatment that exhibited significant differences compared to the control, while the highest was noted with garlic extract (Fig. 6). According to the Bonferroni test, there was no difference between chlorantraniliprole and indoxacarb. Additionally, there were no significant differences between the indoxacarb treatment, the neem and chili treatments, and the control.

Based on the presented results, chlorantraniliprole exhibited the highest efficacy in both years. Using this insecticide resulted in the lowest damage severity and number of larvae per fruit. Compared to the control, plant extracts showed higher efficacy in the first year of the trial, while indoxacarb was equally effective as the plant extracts.

DISCUSSION

The excessive use of synthetic pesticides in Europe is leading to a series of environmental concerns, such as water quality problems [Hüesker and Lepenies 2022], soil contamination [Medić-Pap et al. 2023], which affects soil functions, biodiversity, and food safety overall [Silva et al. 2019]. The intake of raw and cooked vegetables, such as peppers, is one of consumers' most common pesticide exposure routes [Keikotlhaile et al. 2010]. Developing insecticides that precisely target the main pest species is imperative; however, even those active ingredients accumulate in the environment and, over time, exhibit detrimental effects on many non-target organisms [Aktar et al. 2009]. For these reasons, it is essential to identify practical solutions for managing harmful insect species using the safest control methods available. Following the European Union Directive 2009/128/EC [Official Journal of the European Union 2009], the spreading of biological methods based on the sustainable use of pesticides is one of the main objectives aimed at limiting the risks caused by the use of pesticides on the environment and human health. Insecticides based on chlorantraniliprole and indoxacarb exhibit good efficacy in the control of a wide variety of lepidopteran pests [Ghidiu et al. 2009, Vuković et al. 2018, Moustafa et al. 2021]. However, although chlorantraniliprole has low toxicity to mammals, birds, fish, and most soil invertebrates [USEPA 2020], the study conducted by Abdel-Mobdy et al. [2017] revealed its role in pathological parameters of sub-acute and sub-chronic liver, kidneys and protein profile changes in albi-

no rats. Additionally, potentially harmful effects on the development of chicken embryos (*Gallus gallus domesticus* L.) have been reported, even at very low concentrations [Abbas et al. 2018]. It has also been reported to be highly toxic to bees and aquatic invertebrates and persistent in the environment [USEPA 2020]. Similarly, several insecticides with a high pesticide load per hectare, including indoxacarb, have been banned since 2018 [Gensch et al. 2024]. Many formulations containing indoxacarb have been banned in the EU due to unacceptably high risks to bees, beneficial arthropods, birds, and small mammals or because such risks could not be excluded [European Commission 2019, 2021]. However, indoxacarb's approval in biocidal use stretches to December 2026 [Official Journal of the European Union 2024]. It has been shown that several chemical insecticides are difficult to remove from fruits and vegetables. The removal rate of chlorantraniliprole by washing pepper fruits was the lowest (24.8%) among all other applied pesticides, which might be related to its low water solubility [Li et al. 2023].

Plant extracts offer significant advantages in sustainable agriculture and represent a feasible alternative against weeds and pests. Garlic is known for its stimulating properties on plant growth and also protects plants due to its bactericidal and fungicidal activity [Rinaldi et al. 2019]. The demonstrated insecticidal activity of *A. sativum* is based on the presence of allicin [Wanyika et al. 2011]. Aqueous and hydroalcoholic garlic extracts are effective in agriculture [Rinaldi et al. 2019]. Garlic aqueous extract showed a higher efficacy against *Sitophilus zeamais* Motschulsky, which recorded the highest mortality of 98% when comparing its efficacy against *Callosobruchus maculutus* Fabricius, which recorded an 86% mortality. In the first year of our experiment, the garlic solution showed a statistically significant reduction of pepper fruit damage severity and number of ECB larvae in fruits compared to the control and had no significant differences when compared with indoxacarb. Similarly to our findings, Dougoud et al. [2019] reported that in field application, garlic aqueous extracts resulted in a varying level of control of hemipteran, lepidopteran pests, and mites. Compared with positive controls, the efficacy of garlic aqueous extracts was statistically lower in half of the cases [Dougoud et al. 2019].

As a natural compound derived from the neem tree, azadirachtin represents a sustainable alternative to conventional chemical insecticides. During the fruiting of pepper plants, neem-based products should be used to ensure food safety and promote the activities of beneficial arthropods [Adom et al. 2024]. Azadirachtin (0.3% w/w EC) was effective against the false codling moth (FCM) and fruit flies and reduced whitefly populations in chili peppers. Therefore, this promising biopesticide could be used in integrated pest management [Adom et al. 2024]. It has also been reported that at extremely low concentrations of 1 and 10 ppm, azadirachtin proved to be an effective botanical insecticide for controlling *O. nubilalis* [Arnason et al. 1985]. Our results showed that the neem-based treatment in the first experimental year significantly reduced pepper fruit damage severity compared to the control and performed almost equally well as indoxacarb. Gagnon [1992] reported similar findings against the ECB. This author stated that a neem seed kernel extract sprayed before the artificial infestation of the sweet corn plants provided excellent protection against European corn borer damage and significantly reduced larval populations [Gagnon 1992]. Meisner et al. [1985] reported that they observed no pupation of ECB larvae with fresh residues on sweet corn seedlings of 1.0, 0.5, and 0.25% neem extract; only 7% and 16% of the larvae pupated at 0.1% and 0.05% concentration, respectively. According to Meisner et al. [1985], larval weight on the treated leaves was significantly lower at all observation times than on untreated ones, and no pupation occurred even on 8-day-old residues. The high and consistent efficacy of neem-based products against many insect pests could be explained by the fact that the active ingredient azadirachtin possesses multiple modes of action, including antifeedant, deterrent, and growth disruption effects [Mordue and Nisbet 2000]. However, it has been reported that exceptional weather conditions (high temperatures, dry conditions) may harm the efficacy of foliar-applied neem products [Gagnon 1992]. This statement follows the results obtained in our experiment.

Low toxicity to humans and animals and very low environmental risk make chili pepper extracts promising alternatives to chemical insecticides in sustainable pest management strategies. Capsaicin showed

high efficacy against the green peach aphid *Myzus persicae* Sulzer in pepper [Koleva Gudeva et al. 2013], and chili pepper (*Capsicum frutescens* L.). Aqueous extract demonstrated high insecticidal potential in the control of pink hibiscus mealybug *Maconellicoccus hirsutus* Green. Even at low concentrations, the mortality was reported to be higher than 70% [Marchiori et al. 2023]. In addition to that, caterpillars of *Spodoptera latifascia* Walker (Lepidoptera: Noctuidae) reared on highly pungent fruits of the Habanero variety had longer development time, reduced pupation success and lower adult emergence [Chabaane et al. 2022]. Our study on chili extracts showed no significant efficacy expressed through pepper fruit damage severity, and the number of surviving larvae was compared with the control in any of the two experimental years. According to Dougoud et al. [2019], the results of chili pepper aqueous extract application in field experiments against insect pests from various orders were inconsistent, underlining that further research is needed to draw reliable conclusions.

In our experiment, in both years, the treatment with the highest efficacy expressed through pepper fruit damage severity and the number of surviving larvae was chlorantraniliprole, while indoxacarb did not differ from the control treatment only in 2019 for the number of larvae. The plant extracts and neem oil treatment did not exhibit statistically significant efficacy in 2019. It may be attributed to the abnormally high rainfall at the beginning of August 2019, coinciding with the application of the treatments. Therefore, the above-average precipitation in August 2019 likely negatively affected the persistence of the active ingredients on the pepper leaves and fruit, resulting in reduced efficacy across nearly all treatments. The damage severity scale has proven helpful for a reliable infestation assessment and effective pest management decision-making.

CONCLUSION

While synthetic insecticides such as chlorantraniliprole and indoxacarb are effective against *O. nubilalis* in pepper production, their associated environmental and health risks, environmental persistence, and regulatory restrictions underline the urgent need for safer alternatives. The two-year study results demonstrate that among the tested plant extracts, garlic, and neem-based extracts showed promising efficacy, particularly under favorable weather conditions, and in some cases, performed comparably to indoxacarb. In contrast, chili extract did not significantly reduce fruit damage or larval presence in either experimental year. The damage severity rating scale proved effective for assessing infestation levels and guiding pest management decisions. These findings support the possible integration of plant extract insecticides, particularly garlic and neem, into sustainable pest management strategies, per EU directives on reducing synthetic pesticide use. Further studies are needed to optimize application methods in different agroecological conditions.

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UNDERSTANDING THE IMPACT OF ACETAMIPRID-BASED INSECTICIDES ON THE BIOLOGICAL FITNESS OF ENTOMOPATHOGENIC NEMATODES: IMPLICATIONS FOR BIOLOGICAL CONTROL

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ABSTRACT

The impact of acetamiprid-based insecticides on the survival and activity of entomopathogenic nematodes (EPNs) was evaluated in laboratory, focusing on two species, *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Despite variations in sensitivity, with S. feltiae showing greater susceptibility, both species maintained their ability to infect *Galleria mellonella* larvae after exposure. Exposure to Mospilan 20 SP® significantly decreased the reproductive capacity of S. feltiae ($F = 443.215$, $p < 0.001$), while H. bacteriophora showed greater resilience, especially when exposed to and Kobe 20 SP®. The ED50 values for *H. bacteriophora* increased over time with Kobe 20 SP® (0.46 ±0.04 at 24 h to 0.60 ±0.01 at 96 h), while Mospilan 20 SP® decreased the ED50 for *S. feltiae* (0.55 ±0.02 at 24 h to 0.64 ±0.03 at 96 h). The study highlights that the effects of systemic insecticides extend beyond immediate mortality, influencing reproductive potential and long-term viability, particularly for more sensitive species like *S. feltiae*. These findings raise important considerations for integrating EPNs into pest management strategies, especially in systems reliant on chemical pesticides. Further research is recommended to explore the broader ecological impacts of neonicotinoids on beneficial nematodes and their potential interactions with other biocontrol agents, aiming to enhance the sustainability of integrated pest management systems.s.

Keywords: biocontrol agents, *Heterorhabditis bacteriophora*, pest management, reproductive capacity, *Steinernema feltiae*

INTRODUCTION

Neonicotinoids are active ingredients widely used in plant protection products for pest control [Kundoo et al. 2018]. These systemic pesticides are absorbed by plants and distributed throughout their tissues, targeting the central nervous system of insects and causing paralysis and eventual death [Simon-Delso et al. 2015, Casida 2010]. Neonicotinoids are classified into two main groups: N-cyanoamidines and N-nitroguanidines [Jeschke and Nauen 2008, Ligtelijn et al. 2024]. Acetamiprid,

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a member of the N-cyanoamidines group, is comparatively less studied for its ecotoxicity [Morrissey et al. 2015]. It is primarily used as a foliar insecticide spray, leading to direct exposure of various soil organisms, including entomopathogenic nematodes (EPNs) [El-Ashry et al. 2020, Özdemir et al. 2021]. Although EPNs reside in the soil, foliar insecticide sprays can indirectly impact them. The chemicals from the spray can run off into the soil, affecting the habitat and overall environment of the nematodes. This indirect exposure can influence their survival, behavior, and ability to control pest populations effectively. In integrated pest management (IPM), neonicotinoids, particularly acetamiprid, are commonly used and often combined with biological control agents like EPNs to enhance pest suppression [Özdemir et al. 2020].

EPNs are essential biological control agents for managing soil-dwelling pests and belong to the families *Steinernematidae* and *Heterorhabditidae*. These nematodes infect and kill insect pests during their larval stages through a symbiotic relationship with specific bacteria (*Xenorhabdus* spp. in *Steinernema* and *Photorhabdus* spp. in *Heterorhabditis*). Once the nematodes enter the insect host, they release bacteria that multiply rapidly within the host. The bacteria produce toxins that break down tissues and suppress the insect's immune system, ultimately leading to the host's death within 24–48 hours. Once the insect host dies, the nematodes complete their lifecycle by reproducing inside the cadaver, producing new infective juveniles that are released into the environment to seek out and infect new hosts [Stefanovska et al. 2023]. EPNs are critical components of soil ecosystems, contributing to pest population regulation and maintaining ecological balance.

Given the environmental risks associated with acetamiprid and its extensive application, as well as the role of EPNs as biocontrol agents, it is imperative to evaluate the interactions between EPNs and neonicotinoids. This understanding is crucial to assessing the impact of these pesticides on beneficial soil organisms. Most research to date has focused on imidacloprid and thiamethoxam, members of the N-nitroguanidine class [Polavarapu et al. 2007, Miranda et al. 2016, Koppenhöfer et al. 2020, Koppenhöfer and Foye 2024]. However, comprehensive data on the effects of acetamiprid on EPNs remains scarce.

This study aims to bridge this gap by evaluating the effects of two acetamiprid-based formulations (Mospilan 20 SP® and Kobe 20 SP®) on two EPN species (*Steinernema feltiae* and *Heterorhabditis bacteriophora*). The potential risks of disrupting biological control agents when combining insecticides with IPM strategies are assessed through the following objectives:

1. Evaluate the survival of infective juveniles (IJs) of *S. feltiae* and *H. bacteriophora* after direct exposure to two neonicotinoid insecticides at three concentrations over 24, 48, 72, and 96 hours.
2. Assess the virulence of *S. feltiae* and *H. bacteriophora* IJs against *Galleria mellonella* larvae after exposure to two neonicotinoids at varying concentrations.
3. Investigate the reproductive potential of *S. feltiae* and *H. bacteriophora* on *G. mellonella* following exposure to insecticides.

MATERIAL AND METHODS

This study used two acetamiprid-based neonicotinoids, Mospilan 20 SP® and Kobe 20 SP®, to assess their impact on the survival and performance of *S. feltiae* and *H. bacteriophora*. The insecticides were tested in a controlled laboratory setting using three concentrations – 0.5%, 1%, and 1.5% – based on the manufacturers' recommended doses for pest control. These concentrations were based on the average recommended field doses for neonicotinoids: Mospilan 20 SP at 275 g/ha and Kobe 20 SP at 100 g/ha, which were then converted into the appropriate laboratory concentrations. The application volume was standardized to match typical field conditions, ensuring that the experimental setup reflected the pesticide's use under real conditions. The control treatment (0% insecticide concentration) consisted of distilled water.

Commercial strains of EPNs in the infective juvenile (IJ) stage were employed: *H. bacteriophora* (*B-Green*, Biobest Group NV, Belgium) and *S. feltiae* (*Steinernema-System*, Biobest Group NV, Belgium). These biopreparations were sourced simultaneously, ensuring high quality. The greater wax moth, *G. mellonella*, served as the host insect. The laboratory culture of *G. mellonella* was maintained at 25°C with 60–70% relative humidity under a 16:8 h light/dark

cycle. Larvae were reared according to the protocol described by Stefanovska et al. [2024], ensuring proper development. Healthy fifth instar larvae were selected for inoculation with nematodes. The study consisted of three bioassays, as described in Kaya and Stock [1997].

Study design

Nematode viability. The survival of IJs of *S. feltiae* and *H. bacteriophora* exposed to various concentrations of Mospilan 20 SP ® and Kobe 20 SP ® was evaluated. For each treatment, 1000 IJs were placed in 50 mm Petri dishes containing 5 cm³ of insecticide solution. The dishes were kept at 20°C, and nematode mortality was assessed every 24 hours over four days using a stereomicroscope. Distilled water was used as the control.

Nematode virulence. IJs previously exposed to insecticides (subsection Nematode viability) were rinsed three times with deionized water and transferred to 100 mm Petri dishes containing filter paper. Ten *G. mellonella* larvae were placed in each dish (30 insects per treatment variant, with three replicates per concentration). The ability of the nematodes to infect hosts after exposure to insecticides was recorded. Control samples consisted of IJs exposed to distilled water.

Reproductive potential. To assess reproductive capacity, *G. mellonella* cadavers were placed in dish traps as described by White [1927]. The experiment was conducted at 24°C, observing the migration of infective larvae from newly emerged IJ generations into the aquatic environment. Migration occurred after 10 days for *S. feltiae* and 14 days for *H. bacteriophora*. Experimental conditions were consistent across all treatments and nematode species.

Data analysis

The symmetric log-logistic model was employed to evaluate the response of nematode survival over time under the influence of various doses of insecticides [Vanegas and Paula 2016]. This model is well-suited for analyzing dose-response relationships, as it provides estimates for key parameters such as the lower asymptote (c), the upper asymptote (d), the slope (b), and the median effective dose (ED50). The mathematical representation of the model is as follows:

$$Y = \mathrm{c} + \frac{\mathrm{d} - \mathrm{c}}{1 + exp(\mathrm{b}(\log(\mathrm{x}) - log(ED50)))},$$

where Y is the response, c denotes the lower limit of the response when the dose x approaches infinity, d is the upper limit when the dose x approaches 0, b denotes the slope around the point of inflection, which is the ED50, i.e. the dose required to reduce the response half-way between the upper and lower limit. Nematode virulence (time to infect) was analysed using beta regression (β), while reproductive potential (log-transformed IJ counts) was assessed with a general linear model (GLM) including insecticide, concentration, and species as factors. Calculations were performed using the drc package [Ritz et al. 2015]. Descriptive statistics and GLM parameters were calculated using the Statistica software [StatSoft Inc. 2014].

RESULTS

Impact of insecticides on EPNs viability

The data obtained indicate that the effect of insecticides on nematode mortality depends on both the insecticide brand and the duration of exposure (Fig. 1). Symmetric log-logistic models effectively described the relationship between insecticide concentration and nematode mortality, which is confirmed by the high correspondence of the obtained curves to the experimental data (Table 1). With an increase in the duration of exposure, a decrease in the lower limit of the mortality curves was noted, indicating a decrease in the baseline level of nematode mortality. At the same time, there was a decrease in the slope of the curves, which indicates a decrease in the rate of change in mortality with increasing insecticide concentration. The smallest decrease in the lower limit of mortality was recorded for *H. bacteriophora* after treatment with Kobe 20 SP®, which may indicate increased resistance of this species to this insecticide.

Analysis of ED50 values revealed significant differences between insecticides. In the case of Kobe 20 SP®, an increase in ED50 was observed over time, indicating a decrease in the insecticide effectiveness, probably due to the adaptation of nematodes to the toxic effect. On the other hand, the use of Mospilan 20 SP® led to a decrease in ED50, indicating an increase

Table 1. Parameters of the symmetric log-logistic models of nematode survival response to insecticide exposure depending on exposure times

Parameter	Time (h)	Species					
		Steinernema feltiae			*Heterorhabditis bacteriophora*		
		b ±SE	*t*-value	*P*-level	*b* ±SE	*t*-value	*P*-level
		Kobe 20 SP®					
Slope	24	3.94 ±0.63	6.3	<0.001	5.51 ±6.72	0.8	0.418
	48	3.38 ±0.43	7.9	<0.001	3.07 ±0.74	4.2	<0.001
	72	2.45 ±0.30	8.2	<0.001	5.01 ±0.86	5.8	<0.001
	96	2.69 ±0.25	10.8	<0.001	3.06 ±0.20	15.2	<0.001
Lower limit	24	0.67 ±0.01	72.1	<0.001	0.92 ±0.00	257.2	<0.001
	48	0.62 ±0.01	54.6	<0.001	0.86 ±0.01	138.9	<0.001
	72	0.53 ±0.02	26.9	<0.001	0.77 ±0.00	204.9	<0.001
	96	0.47 ±0.02	26.6	<0.001	0.64 ±0.01	95.2	<0.001
Upper limit	24	1.00 ±0.01	172.3	<0.001	1.00 ±0.00	347.4	<0.001
	48	0.99 ±0.01	170.7	<0.001	0.99 ±0.00	345.7	<0.001
	72	0.98 ±0.01	170.1	<0.001	0.98 ±0.00	340.5	<0.001
	96	0.95 ±0.01	164.6	<0.001	0.96 ±0.00	336.0	<0.001
ED50	24	0.55 ±0.02	31.8	<0.001	0.46 ±0.04	10.3	<0.001
	48	0.57 ±0.02	29.3	<0.001	0.50 ±0.02	22.7	<0.001
	72	0.57 ±0.03	21.2	<0.001	0.53 ±0.01	51.0	<0.001
	96	0.64 ±0.03	24.0	<0.001	0.60 ±0.01	43.5	<0.001

Mospilan 20 SP®							
Slope	24	3.60 ±1.44	2.5	0.018	3.94 ±0.63	0.81 ±0.82	1.0
	48	2.32 ±0.55	4.2	<0.001	3.38 ±0.43	0.59 ±0.50	1.2
	72	1.34 ±0.43	3.1	0.004	2.84 ±0.30	0.45 ±0.27	1.6
	96	2.25 ±0.58	3.9	0.001	2.69 ±0.25	0.51 ±0.30	1.7
Lower limit	24	0.76 ±0.01	67.4	<0.001	0.67 ±0.01	0.46 ±2.53	0.2
	48	0.60 ±0.02	27.8	<0.001	0.62 ±0.01	0.45 ±1.42	0.3
	72	0.46 ±0.07	6.8	<0.001	0.53 ±0.02	-0.11 ±1.64	–0.1
	96	0.46 ±0.02	21.5	<0.001	0.49 ±0.02	-0.31 ±2.03	–0.2
Upper limit	24	1.00 ±0.01	152.6	<0.001	1.00 ±0.01	1.00 ±0.02	41.7
	48	0.99 ±0.01	152.1	<0.001	0.99 ±0.01	0.99 ±0.02	41.6
	72	0.99 ±0.01	151.9	<0.001	0.98 ±0.01	0.98 ±0.02	41.3
	96	0.99 ±0.01	151.6	<0.001	0.95 ±0.01	0.98 ±0.02	41.2
ED50	24	0.47 ±0.02	25.1	<0.001	0.55 ±0.02	7.40 ±63.63	0.1
	48	0.46 ±0.02	21.7	<0.001	0.56 ±0.02	7.46 ±52.73	0.1
	72	0.43 ±0.07	6.1	<0.001	0.57 ±0.03	7.57 ±44.25	0.2
	96	0.37 ±0.02	20.8	<0.001	0.64 ±0.03	6.42 ±34.67	0.2

b – the slope coefficient of the model, indicating the steepness of the dose-response curve; SE – standard error

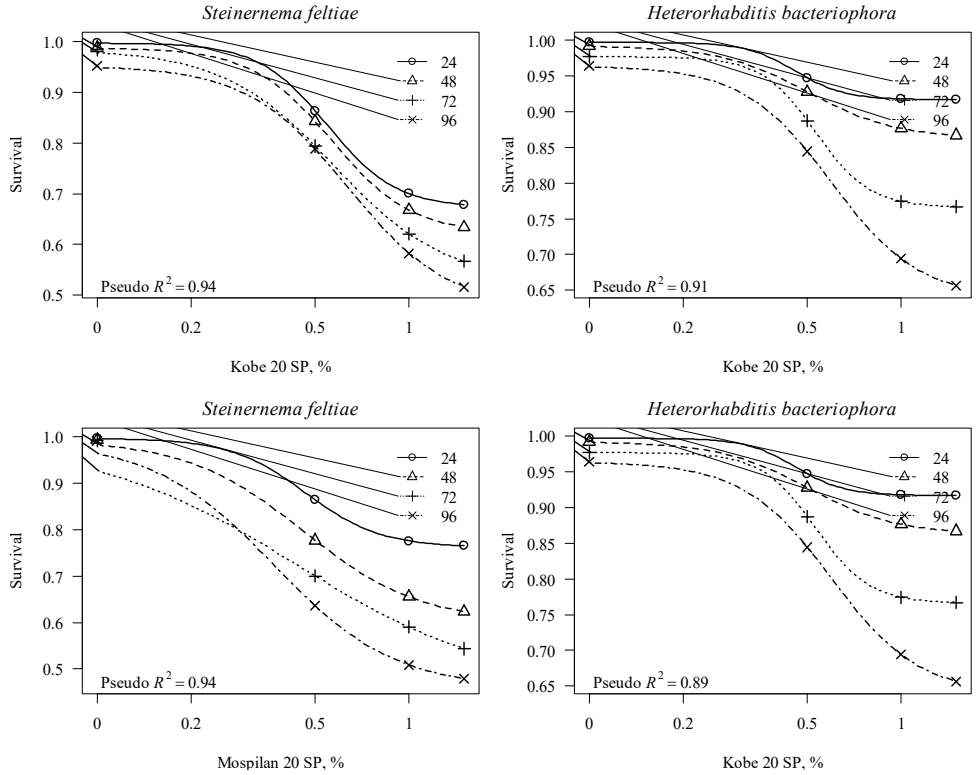

Fig. 1. The symmetric log-logistic models of the response of nematode (IJs) survival to insecticide exposure depending on exposure time (24, 48, 72, and 96 hours). The ordinate axis is the concentration of insecticides, the abscissa is the proportion of surviving IJs

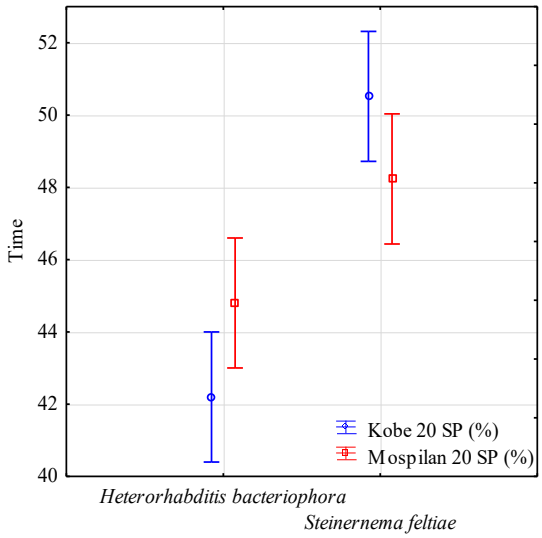

Fig. 2. Variation in the time of infection of G. mellonella by entomopathogenic nematodes previously stored in solutions of the tested insecticides (Mospilan 20 SP® and Kobe 20 SP®) at concentrations of 0.5%, 1%, and 1.5%. The dose of pesticides was used as a covariate in the analysis

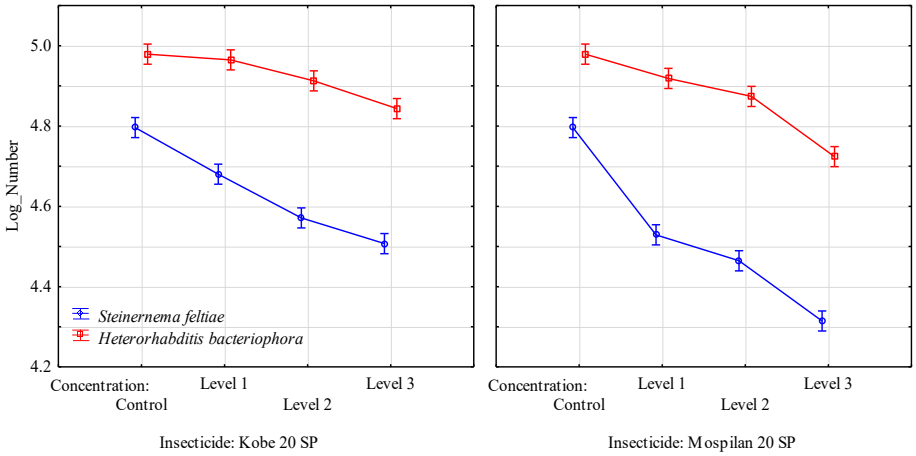

Fig. 3. Reproductive capacity of EPNs depending on the applied insecticide. The x-axis represents the applied pesticide: Mospilan 20 SP® or Kobe 20 SP®, and the species of nematodes: *H. bacteriophora* or *S. feltiae*. Level 0 corresponds to the control (0% insecticide), while Levels 1, 2, and 3 correspond to concentrations of 0.5%, 1.0%, and 1.5%, respectively. The y-axis represents the log-transformed number of infective juveniles (IJs)

in insecticide efficacy and raising nematode mortality even at lower doses.

Effect of insecticides on *Galleria mellonella* virulence

Entomopathogenic nematodes (EPNs) demonstrated the ability to significantly delay the infection process in the greater wax moth larvae (Fig. 2). In the control group, infection of *G. mellonella* occurred within 35.2 ±0.3 hours. The inhibitory effects of pesticides on nematode virulence were species-specific and dose-dependent ($R_{adj}^2 = 0.89$, $F = 82.4$, $p < 0.001$). However, within the tested dose ranges, the insecticides did not differ significantly in their overall impact on infection rates by nematodes ($F = 0.04$, $p = 0.84$).

Application of Mospilan 20 SP® led to stronger inhibition of virulence in *H. bacteriophora* compared to *S. feltiae* (Planned comparison $F = 4.2$, $p = 0.04$). In contrast, Kobe 20 SP ® exhibited no significant species-specific inhibitory effect on infection rates (Planned comparison $F = 3.3$, $p = 0.08$). Increasing pesticide doses significantly delayed the infection of *G. mellonella* by nematodes ($\beta = 0.88 \pm 0.05$, $t = 18.7$, $p < 0.001$).

The sensitivity of nematodes to pesticide doses varied between species. Delays in infection caused by *H. bacteriophora* at increasing pesticide doses were less pronounced compared to those caused by *S. feltiae* (β for species * dose interaction = –0.28 ±0.08, t = –3.5, $p = 0.001$). At the highest tested pesticide concentration, *H. bacteriophora* infected *G. mellonella* within 51.2 ±1.2 hours, whereas *S. feltiae* required 61.9 ±1.0 hours.

EPN reproductive capacity

The reproductive potential of entomopathogenic nematodes, measured as the total number of infective juveniles (IJs) emerging from *G. mellonella* cadavers, varied significantly based on experimental factors (Fig. 3). Nematode species, insecticide type, and concentration collectively accounted for 86% of the variation in reproductive capacity ($R_{adj}^2 = 0.86$, $F = 611.6$, $P < 0.001$) (Table 2).

A significant reduction in the reproductive capacity of *S. feltiae* was observed, particularly following exposure to Mospilan 20 SP® (Planned comparison $F = 443.215$, $p < 0.001$). Increasing concentrations of both tested insecticides led to a substantial reduction in the reproductive capacity of nematodes (β for concentration = –0.46 ±0.02, t = –30.9, $p < 0.001$).

The effects of increasing pesticide concentrations were less pronounced for *S. feltiae* compared to *H. bacteriophora* (β for concentration * species interaction = 0.07 ±0.01, t = 4.5, $p < 0.001$). For *H. bacteriophora*, the number of larvae (IJs) emerging

Table 2. General linear model (GLM) of the effect of insecticides, insecticide concentration, and species on the EPNs reproductive capacity

Source of variation	Sum of squares	df (degrees of freedom)	Mean sum of squares	*F*-ratio	*p*-value
Intercept	4085.2	1	4085.2	663757.1	<0.001
Insecticide	0.8	1	0.8	130.7	<0.001
Species	2.2	1	2.2	355.1	<0.001
Insecticide × species	0.1	1	0.1	18.4	0.002
Concentration (continuous)	5.3	1	5.3	859.2	<0.001
Species × concentration	0.6	1	0.6	90.1	<0.001
Error	2.9	474	0.0	–	–

from insect cadavers remained similar to control levels, particularly following exposure to Kobe 20 SP®. This suggests that *H. bacteriophora* exhibited greater resilience to pesticide exposure compared to *S. feltiae*.

DISCUSSION

Soil fauna encompasses a rich diversity of taxonomic groups, including nematodes [Ramirez et al. 2015]. Intensive agricultural and horticultural practices often rely on chemical pesticides to protect crops; however, these pesticides can adversely affect beneficial organisms such as EPNs. Entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae play a crucial role in regulating pest populations and are widely used as biocontrol agents. However, pesticide contamination in soils can compromise EPN survival, reproduction, and efficacy in pest control, with responses varying depending on pesticide type, concentration, and nematode species [Ulu 2023].

In this study, the effects of two acetamiprid-based neonicotinoids (Mospilan 20 SP® and Kobe 20 SP®) on *S. feltiae* and *H. bacteriophora* were evaluated. Our findings revealed varying sensitivity among the two EPN species, with *S. feltiae* exhibiting greater sensitivity to acetamiprid exposure compared to *H. bacteriophora*. Significant reductions in fecundity and reproductive potential were observed for both species, although the ability to infect the insect host *G. mellonella* remained largely unaffected.

These results align with previous studies, which reported species-specific tolerances to pesticides among EPNs [Ulu et al. 2016, Kruk and Dzięgielewska 2020]. While acetamiprid exposure reduced fecundity, the virulence of *S. feltiae* and *H. bacteriophora* remained unchanged. This suggests that acetamiprid primarily affects reproduction rather than the infection capability of EPNs.

Interestingly, studies on imidacloprid, another neonicotinoid, have shown more complex interactions with EPNs. For example, imidacloprid has been reported to enhance the efficacy of *Steinernema* species in some cases by promoting host attraction or infection rates [Atwa et al. 2013]. Synergistic effects have also been observed between imidacloprid and *Steinernema* spp., wherein the pesticide increases nematode efficiency in pest control. However, our study revealed that acetamiprid causes higher nematode mortality than imidacloprid, a finding consistent with previous research on EPN compatibility with these pesticides [Koppenhöfer and Grewal 2005]. Greenhouse and field studies on scarab larvae demonstrated that imidacloprid enhanced EPN efficacy, whereas acetamiprid and thiamethoxam exhibited weaker interactions.

Koppenhöfer et al. [2002]. reported that imidacloprid has stronger adverse effects on entomopathogenic nematodes than acetamiprid, but our results suggest that acetamiprid has more persistent negative effects

on *S. feltiae* reproductive capacity. This limitation could reduce the long-term biocontrol potential of *S. feltiae*. The higher toxicity of acetamiprid, compared to imidacloprid, may result from differences in their chemical structures, modes of action, or toxicity profiles. Acetamiprid appears to have a more potent effect on nematode fecundity by regulating immune pathways, as previously suggested in related studies.

The effects of pesticides on EPNs are also influenced by exposure duration and nematode species. For instance, Laznik and Trdan [2014] demonstrated that prolonged exposure to chemical solutions exacerbates nematode sensitivity. Our findings support these observations, as exposure duration significantly influenced EPNs survival and reproduction.

Species-specific sensitivity was also evident in this study. *S. feltiae* exhibited lower resistance to acetamiprid compared to *H. bacteriophora*. This suggests that *S. feltiae* may be more vulnerable to pesticide contamination in agricultural soils where acetamiprid is commonly applied. Conversely, the higher resistance of *H. bacteriophora* indicates its potential as a more robust biocontrol agent in environments with acetamiprid exposure.

CONCLUSIONS

The results showed that *S. feltiae* was more vulnerable to acetamiprid than *H. bacteriophora*, with significantly higher mortality rates observed in *S. feltiae* after four days of exposure. However, pesticide exposure did not significantly affect the nematodes' ability to infest *G. mellonella*, suggesting their parasitizing ability remained intact.

Steinernema feltiae also exhibited reduced fecundity and reproductive capacity after exposure, indicating lower resistance compared to *H. bacteriophora*. This raises concerns about the long-term viability of *S. feltiae* in areas with frequent pesticide use. The study highlighted that EPN species can show different sensitivities to the same insecticide, complicating their use in integrated pest management (IPM) systems involving chemical pesticides.

Further research is needed to assess the long-term impacts of acetamiprid and other neonicotinoids on EPN reproductive capacity and population dynamics, particularly *S. feltiae* and *H. bacteriophora*. Such studies are essential for evaluating their sustainability as biocontrol agents in pest management. Additionally, exploring potential synergistic interactions between neonicotinoids and other biocontrol agents, such as fungi or bacteria, could improve pest control while minimizing pesticide impact on EPN populations. This research will be crucial for developing sustainable pest management strategies that protect both crops and beneficial soil organisms.

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