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Yield and fruit quality response to foliar application of biostimulants in an apple orchard

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Summary: Nutrient supply plays an important role in fruit production technology. Among the methods of nutrient management, the application of foliar fertilization has become an essential tool to harmonize the vegetative and generative performance of fruit trees. This study was conducted to determine the effect of four foliar fertilizers (Bistep, brown juice, *Trichoderma harzianum*, *Clonostachys rosea*) on yield and fruit quality in an apple orchard with the cultivar 'Pinova', at the University of Debrecen in Hungary. Trees were trained to a slender spindle canopy with a spacing of 4 × 1 m. Treatments were applied five times during different phenological phases in 2021 and 2022. Based on our results, biostimulants had a positive effect on fruit yield and fruit quality, but extreme weather events also influenced the data. In 2021, the effect of the treatments on yield data could not be observed (10.1–13.5 kg/tree; 0.16–0.24 kg/cm²). However, in 2022, plants sprayed with Bistep, *T. harzianum*, and *C. rosea* reached 28.7–31.2 kg/tree (0.33–0.46 kg/cm²), while control trees produced only 19.8 kg/tree (0.20 kg/cm²). Fruit size development improved with an increase of 2–6 mm over the two years. Among the treatments, Bistep was able to enhance fruit surface coloration to a greater extent in 2021, as red skin color reached 44% for this foliar fertilizer, while control trees presented only 27%.

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Key words: nutrient supply, biostimulants, apple orchard, fruit yield, fruit quality

Introduction

In order to meet the increasing food demand of the world's human population under changing environmental, social, and climatic conditions, agricultural production needs to implement shifts in production technology (Vangenechten et al., 2025). Biostimulants are widely used vital components in modern fruit production practices, offering significant benefits. These compounds, regardless of their natural or synthetic origin, facilitate advantageous outcomes in plant vegetative and generative development by augmenting metabolic activities and enhancing resilience to biotic and abiotic stressors, consequently increasing both agricultural yield and quality (Kauffmann et al., 2007; Kincses et al., 2007; Nagy et al., 2019; de Pascale et al., 2017; Li et al., 2022). Furthermore, they play a constructive role in promoting soil vitality, thereby amplifying their influence on agricultural efficacy (Colla et al., 2017; Arya et al., 2024). Indeed, the term 'biostimulant' emerges as a versatile designation for any agent that confers advantages to plants, excluding nutrients, pesticides, or soil amendments (du Jardin, 2015).

The ascomycete fungus *Clonostachys rosea* was identified by Barnett & Lilly (1962) as a mycoparasite and was subsequently introduced as a biological control agent for phytopathological diseases within a short time (Shigo, 1958; Sun et al., 2020). *C. rosea* is highly competitive for resources (mainly nutrients) and space, acts as a mycoparasite with antibiotic properties (Sutton et al., 1997; Saraiva et al., 2020; Jensen et al., 2021). Furthermore, as an endophyte, it can colonize potential infection points for plant pathogens and induce systemic resistance (Lahoz et al., 2004), while also producing secondary metabolites with antibiotic effects on pathogens (Han et al., 2020). The fungus not only affects microorganisms but also acts as a suppressor of various nematodes, insects, and bugs (Sutton et al., 1997; Rodríguez-Martínez et al., 2018; Sun et al., 2020; Al-Nabhani et al., 2024).

Nanomaterials used in agricultural production promote crop productivity by effectively targeting inputs and nutrients in plants (Chittibomma et al., 2023; Hussain et al., 2023). Plants adapt better to unfavorable conditions such as drought, high temperatures, and non-optimal soil conditions (Chittibomma et al., 2023). These technologies enable producers to apply inputs at the most effective dosage without harming sensitive ecosystems (Vermeulen et al., 2012; Mukhopadhyay, 2014; Prasad et al., 2014; Prasad et al., 2017; Shang et al., 2019). Bistep is a nanotechnology-based biostimulant produced from natural ingredients (Daragó & Kalydi, 2023), containing extracts of wormwood, microorganisms, humic and fulvic acids, as well as macro- and microelements (Csihon et al., 2021a; Daragó & Kalydi, 2023; Kovács et al., 2024). Due to its composition, it has beneficial effects on vegetative parameters, positively influences evapotranspiration and transpiration rates, and reduces abiotic stress effects (Kovács et al., 2024). Furthermore, increases in crop yield, fruit size, and surface coloration have also been observed in apples (Csihon et al., 2021b).

Brown juice, also referred to as deproteinized plant juice, is a liquid by-product from leaf protein production (Pirie, 1990; Bákonyi et al., 2025). This substance contains sugars, oligopeptides, nutrients, biologically active compounds, pigments, vitamins, enzymes, minerals, and phytochemicals (Pirie, 1990; Jadhav, 2018; Bákonyi et al., 2020; Barna et al., 2022; Domokos-Szabolcsy et al., 2023). It is suitable for use in animal husbandry, plant nutrition as a foliar fertilizer, and soil stabilization as a microbial stabilizing medium (Bákonyi et al., 2020; Kisvarga et al., 2020).

Trichoderma harzianum, among other *Trichoderma* species, is widely used as a microbial plant biostimulant and symbiont, providing benefits for both plants and soil (Harman, 2000; Harman et al., 2004). It is capable of counteracting plant fungal pathogens (Bigirimana et al., 1997) and inducing systemic resistance or systemic acquired resistance (Elad, 1994; De Meyer et al., 1998; Shoresh et al., 2010) against phytopathogens by releasing elicitors such as peptides, proteins, and low-molecular-weight compounds (López-Bucio et al., 2020). The symbiotic effect induces plant growth, germination, root development, nutrient solubilization and assimilation, as well as increased resistance to abiotic stresses such as drought, salinity, or extreme temperatures. The interaction between fungus and plant triggers the release of hormones, oligopeptides, volatiles, and other secondary metabolites into the rhizosphere, enhancing root development and nutrient uptake efficiency, thereby promoting crop performance (Siddiqui et al., 2008; Naik et al., 2019; Visconti et al., 2020). *Trichoderma* treatments can be applied not only through soil but also to fruits, flowers, and foliage (Altomare et al., 1999).

The aim of the study was to determine the effect of four biostimulant materials (Bistep, brown juice, *Trichoderma harzianum*, *Clonostachys rosea*) on fruit yield and selected fruit quality parameters in an intensive apple orchard with the cultivar ‘Pinova’.

Materials and methods

Location and training system

A two-year study (2021-2022) was conducted at the Horticultural Experimental Farm of University of Debrecen, in Hungary. Orchard soil type was light sandy loam, humus content was between 1.2-1.6%. The pH of the soil was slightly alkaline (pH 7.5-7.6), the “Arany” number of heaviness was 26-28.

The experimental apple orchard was established in 2006. The trial was performed on cultivar ‘Pinova’. Trees were grafted on M.9 rootstock and designed with spacing of 4 x 1 m (2500 trees/ha). As a training system, slender spindle was created with tree height of 3.5 m. Plantation is equipped with drip irrigation system. Orchard management followed the European Integrated Fruit Production guidelines.

Applied treatments

The experiment consisted of five treatments (control, Bistep, brown juice, *Trichoderma harzianum*, *Clonostachys rosea*). Trees were sprayed with backpack sprayer five times each year, each treatment consisted of 10 trees. Dates of the treatments are presented in ***Table 1***.

Table 1: Phenophases in apple cultivar ‘Pinova’ (Debrecen-Pallag, 2021-2022).

	Green bud stage	Full bloom	3 weeks after full bloom	6 weeks after full bloom	9 weeks after full bloom
2021	8/4	3/5	25/5	11/6	6/7
2022	13/4	29/4	17/5	7/6	8/7

Control trees were sprayed with water only, without active ingredients. Bistep is a nanotechnology-based biostimulant material, and based on our previous study (Csihon et al., 2021a), we applied 3% concentration for tree spraying. Brown juice is an alfalfa-based organic biostimulant, which is a by-product of leaf protein concentrate (Fári & Domokos-Szabolcsy, 2019) and in our trial alfalfa brown juice was used with 2% concentration. *Trichoderma harzianum* is an endophyte microbe isolated from white grapevine plants (cv. ‘Furmint’) from the Tokaj Wine Region, Northeast Hungary (Kovács et al., 2021; Csótó et al., 2024) and was applied at a concentration of 1.0×10^5 spores/mL. *Clonostachys rosea* is a saprophytic filamentous fungus with strong biological control activity against various fungi, nematodes, and insects (Sun et al., 2020), and was also applied at a concentration of 1.0×10^5 spores/mL.

Assessed parameters

During harvest, fruit yield (kg/tree) was measured for each tree. For better comparability, these data were calculated also to the trunk thickness and expressed as crop load (kg/cm^2). Fruit quality parameters (diameter, weight) were assessed based on 100 fruits/treatments. Fruit surface color was evaluated with a visual estimation in percentage form. The intensity (darkness) of the fruit surface color was assessed with similar method on a scale ranging from 1-5, where fruits with lower color intensity were given a value of 1, while fruits with darker skin color were given a value of 5.

In the spring of 2021, the experimental plantation was significantly affected by cold weather, as radiation frosts caused yield damage mainly in the lower zone of the trees. In 2022, extreme summer drought was a significant challenge in achieving the appropriate yield and quality.

Results

In the first year of the experiment, in 2021 harvested fruit yield was between 10.1 and 13.5 kg/tree, which is lower than expected, due to the spring frosts in April (***Figures 1-2***). Control trees produced an average of 12.5 kg of fruits. Larger amount was harvested from the trees treated with brown juice (13.5 kg/tree), while less was picked from the plants sprayed with *T. harzianum* (10.9 kg/tree) and Bistep (10.1 kg/tree). In 2021, fruit number per tree varied up 81 to 112. Consistent treatment effect can not be observed in the yield data, which were influenced more by vintage effects than by the spraying carried out. This is also confirmed by crop load data (***Figures 3-4***), as fruit amount calculated to trunk thickness (TCSA) showed 0.16-0.24 kg/cm^2, 1.35-1.89 fruit number/TCSA.

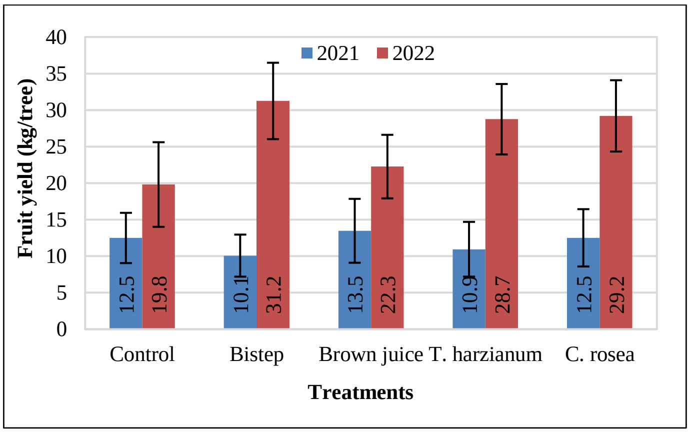

Figure 1. Fruit yield per tree (Debrecen – Pallag, 2021-2022).

In 2022, yields per tree were significantly higher (19.8-31.2 kg/tree) than in the previous year (***Figures 1-2***). The lowest fruit amount was shown by control trees (19.8 kg/tree), while the highest values were observed on the plants sprayed with Bistep (31.2 kg/tree). The yields of trees treated with *T. harzianum* and *C. rosea* approached the values of Bistep treatment (28.7-29.2 kg/tree), while the application of brown juice biostimulant resulted in lower yields (22.3 kg/tree). Fruit number per tree was between 133 and 210. In terms of crop load, *C. rosea* (0.46 kg/cm^2; 2.96 number/TCSA) and Bistep (0.42 kg/cm^2, 2.68 fruit number/TCSA) treatments reached higher values, while the control (0.20 kg/cm^2) and brown juice (0.22 kg/cm^2) treatment showed lower crop load (***Figures 3-4***).

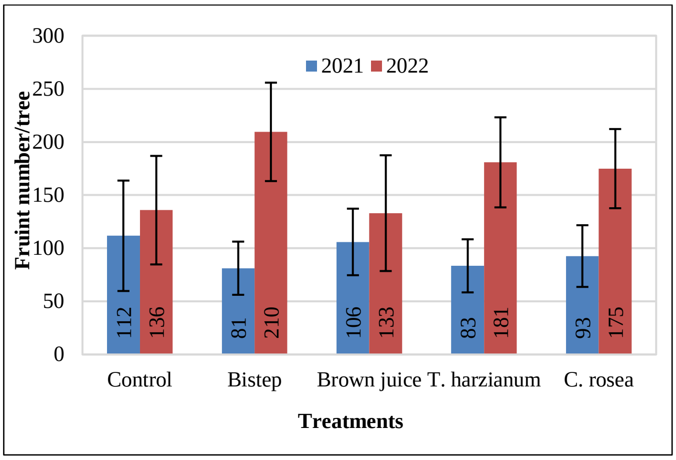

Figure 2. Fruit number per tree (Debrecen – Pallag, 2021-2022).

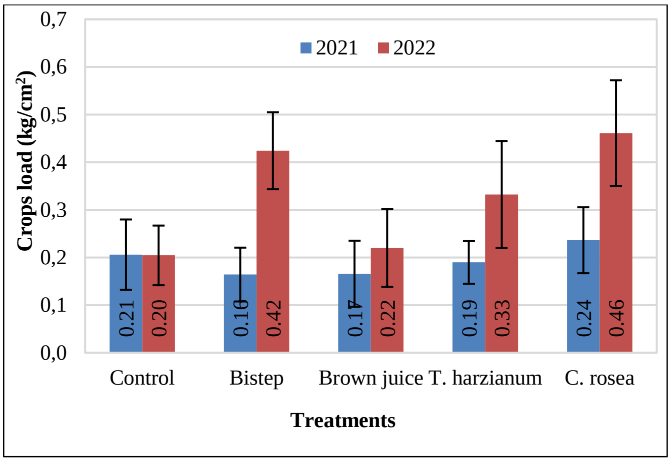

Figure 3. Crop load per tree (kg/cm^2) (Debrecen – Pallag, 2021-2022).

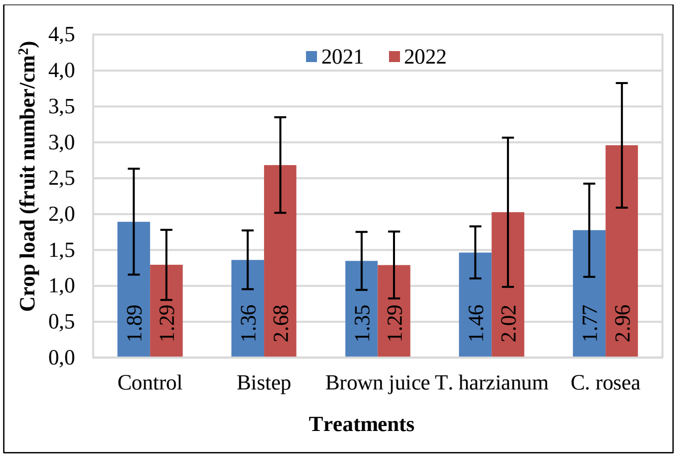

Figure 4. Crop load per tree (fruit number/cm^2) (Debrecen – Pallag, 2021-2022).

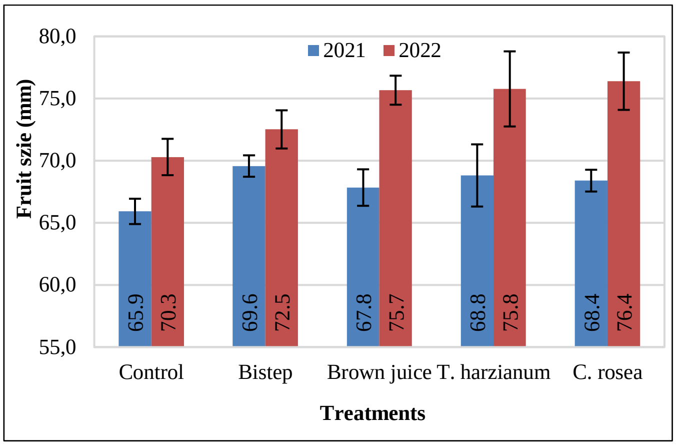

Figure 5. Fruit size (Debrecen – Pallag, 2021-2022).

Among the fruit quality attributes, the most important factor affecting the marketability of apples is their size. Despite the low crop load per tree in 2021, diameter of the fruits did not reach 70 mm size expected by the fresh market (***Figure 5***). The smallest diameter was recorded on the control trees (65.9 mm), while the largest was on the trees treated with Bistep (69.6 mm). The size of the fruits treated with the other three preparations also exceeded the control (67.8-68.8 mm, so an increase in size of around 2-3 mm was observed as a result of the treatments.

In 2022, fruit size exceeded 70 mm for all treatments. The smallest diameter was measured in the control trees (70.3 mm) and the largest in the *C. rosea* treatment (76.4 mm). The fruit size of the brown juice and *T. harzianum* treatments was the same (75.7-75.8 mm), while trees sprayed with Bistep reached 72.5 mm diameter. As we have seen previously, crop load was the highest for *C. rosea* and Bistep treatments in 2022, but the fruit diameter also satisfied the requirements of the fresh market. Eventually, biostimulants had a positive effect on fruit size development, with an increase in diameter between 2 and 6 mm.

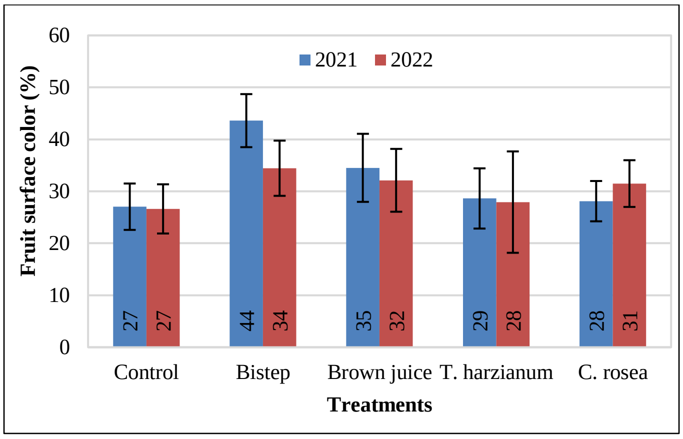

Figure 6. Fruit surface color (Debrecen – Pallag, 2021-2022).

The marketability of the fruits is highly influenced by the degree of fruit surface coloration. For bicolour cultivars, the market requires a minimum of 50-70% red skin color. Cultivar 'Pinova' did not reach adequate coloration in none of the years observed due to the unfavourable weather conditions (***Figure 6***). In 2021, the lowest value was recorded on the control trees (27%), as the two endophytic fungi treatment showed practically the same results (28-29%). Application of brown juice was able to enhance coloration to a lesser extent (35%), while the most colourful fruits were obtained on plants treated with Bistep (44% fruit surface coloration), which can be considered as a significant improvement compared to the control.

In the year 2022, there were minor differences between the biostimulants, but the trend was similar, as Bistep treatment presented the highest red skin color (34%), resulting 7% difference compared to control trees. Other biostimulants showed an improvement in coloration of 1-5%.

Fruit color intensity is also an important quality parameter in terms of saleability. Control trees showed the lowest values (2.9) on a scale of 1-5 in both years (***Figure 7***). In 2021, a slight improvement was observed for the two endophytic fungi and for brown juice (3.2). The darkest colored fruit (4.3) was obtained from trees sprayed with Bistep. In 2022, the most significant improvement was measured for the *C. rosea* treatment (3.6), while fruit color intensity values for the other biostimulants ranged from 3.0 to 3.3.

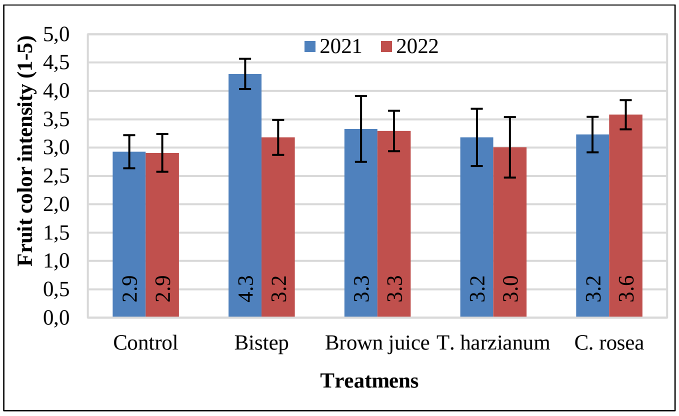

Figure 7. Fruit color intensity (Debrecen – Pallag, 2021-2022).

Discussion

Biostimulants play an important role in plant nutrition management. Several studies have shown that the use of biostimulants can be an effective way to improve fruit yield and quality (Soppelsa et al., 2018; Nagy et al., 2019; Graziani et al., 2020; Mosa et al., 2023). Moreover, various authors have reported that the application of biostimulants can help mitigate damage caused by biotic stresses (Lötze & Hoffman, 2014; Zargar, 2019; Froni et al., 2021). However, it should be noted that the effects of biostimulants have limitations, and their use must be combined with other techniques to maximize fruit yield and quality under current climatic conditions (Zulfiqar et al., 2024).

In this study, four foliar fertilizers (Bistep, brown juice, *T. harzianum*, and *C. rosea*) were evaluated in an intensive apple orchard. According to the results, the application of the nanotechnology-based Bistep increased the crop load of apple trees and improved fruit size and surface color. In our previous research, Bistep treatment also enhanced red skin color development in apples (Csihon & Gonda, 2017, 2018), led to earlier harvest and higher water-soluble dry matter content in sour cherry (Csihon & Gonda, 2016), increased fruit diameter and weight in plum (Csihon & Gonda, 2020), and resulted in higher fruit set and earlier harvest in table grapes (Csihon et al., 2021b). In the study by Yaseen & Takácsné Hájos (2021), Bistep application improved the vegetative parameters of lettuce. In ornamental plant production, it also had a positive effect by stimulating root formation in *Pelargonium* (Tilly-Mándy et al., 2011) and enhancing shoot development in *Petunia × grandiflora* (Kisvarga et al., 2014).

In the present study, brown juice had no significant effect on fruit yield or crop load, but it improved fruit quality in the 'Pinova' apple cultivar. In an ornamental plant trial, applying 0.5% fermented brown juice significantly improved the seed germination of marigold (*Tagetes patula* L.) and increased root and shoot length (Barna et al., 2021). In a sweet basil (*Ocimum basilicum* L.) experiment, foliar application of alfalfa brown juice significantly improved biometric parameters (Kisvarga et al., 2020). However, in some cases, the use of fermented brown juice has been reported to reduce crop development and yield (Ream et al., 1977).

In our trial, *T. harzianum* increased crop load only in 2022, during drought conditions, and improved fruit size. These results are consistent with those of Csótó et al. (2024), who found that foliar treatment with *Trichoderma* spores increased maize yield under extreme drought. In another study, Soltaniband et al. (2022) reported improved internal fruit quality in strawberries in response to *Trichoderma* application.

In this research, treatment with *C. rosea* resulted in higher yield and crop load, and positively affected fruit size presumably by increasing the plant's tolerance to pathogens and improving overall plant condition. In strawberry production, spraying with *C. rosea* led to higher yields by protecting plants against grey mold (*Botrytis cinerea*) (Cota et al., 2009). In raspberry production, *C. rosea* treatment was also effective in controlling pathogens (Yu & Sutton, 1997; Morandi et al., 2000).

Conclusions

In our two-year study, fruit yield and fruit quality parameters were influenced by the applied biostimulant treatments, but extreme weather events also affected the given data. Regarding fruit yield, consequent effect of the treatments was found only in the second year of the trial, in 2022, as Bistep and *C. rosea* biostimulants presented 0.42-0.46 kg/cm^2 crop load, while control trees showed only 0.20 kg/cm^2. Fruit size improved in both years, as 2-3 mm diameter increase was observed in 2021, while the rate of the improvement was higher in 2022 with 2-6 mm increase. Among the biostimulants, Bistep had also notable positive effect on red skin color development, as fruit surface color reached 44%, while control trees showed only 27% in 2021. Accordingly, the applied treatments can be effective tool to improve fruit yield and fruit quality in an intensive apple orchard.

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