Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 1, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

# RESEARCH ON THE EFFECTIVENESS OF HYDROGEN PEROXIDE AND AZOXISTROBIN TREATMENTS IN THE ATTACK OF *Fusarium* spp. IN STRAWBERRY CROP

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***Abstract***

Fusarium spp. *infections in strawberries result in significant damage, including stunting, wilting and plant collapse. The efficacy of a 35% hydrogen peroxide solution and the azoxystrobin substance in controlling Fusarium* spp. *fungi on strawberries in Giurgiu County was investigated during the years 2022-2023, using the Alba strawberry genotype. The treatment variants tested were: control (V1); pre-planting immersion of strawberry stolons in 35% hydrogen peroxide solution and drip application of azoxystrobin during the growing season (V2); application of azoxystrobin substance by dripping during the vegetation period (V3) and pre-planting immersion of strawberry stolons in 35% hydrogen peroxide solution (V4). The results showed that V2 resulted in the lowest degree of attack by Fusarium* spp.*, at 1.27%, followed by (V3), at 3.56%. The highest effectiveness (94.5%) was observed for V2, followed by V3, at 83.46%. Therefore, it is recommended that pre-planting immersion of strawberry stolons in 35% hydrogen peroxide solution and drip application of azoxystrobin during the growing season be considered for the management of Fusarium* spp. *infections in the Alba genotype.*

***Key words****: strawberry, Fusarium* spp.*, hydrogen peroxide, azoxystrobin, efficacy.*

## INTRODUCTION

Worldwide, the commercially grown strawberry (*Fragaria* × *ananassa*), is a significant berry fruit crop in terms of economic impact. Being abundant in vitamins, minerals, antioxidants, anthocyanins, and other nutrients, it is strongly suggested as a nutritious diet for people (Warner et al., 2021).

Global strawberry production was estimated at $14 billion in 2020 (FAO, 2021). In Romania, by 2026, it's estimated that the country will produce approximately 25,270 metric tons of strawberries, marking a 0.7% increase from the 24,400 metric tons recorded in 2021. Since 1966, Romania has seen a consistent upward trend in strawberry production, averaging an annual growth rate of 1.2%. In the 2021 global rankings, Romania was positioned 27th, with Chile leading the production at 24,400 metric tons, followed by the US, Mexico, and Egypt as the major producers (www.reportlinker.com/clp/country/2878/726388).

The susceptibility of strawberries to diseases such as *Fusarium* spp. represents a significant challenge for farmers. *Fusarium* spp. infections in strawberry crops are severe, ranging from stunted growth and wilting to the collapse of entire plants (Joshi, 2018). Wilt in strawberries occurs mostly during seedling growth, blooming, and harvesting, with seedlings and soil being the major causes of its recurrence (Yanget et al., 2023). Beyond the visible symptoms, *Fusarium* spp. can compromise the plant's vascular system, leading to decreased nutrient uptake and overall physiological stress. As a result, *Fusarium* spp. induced diseases contribute to economic losses, reduced fruit quality, and compromised sustainability of strawberry cultivation (Pastrana et al., 2023).

*Fusarium* spp. induced diseases are notorious for their insidious onset, often remaining asymptomatic during initial stages and becoming apparent only when the infection is well-established. This stealthy nature underscores the importance of proactive management strategies to curb the impact of *Fusarium* spp. on strawberry production.

Consequently, there is a growing emphasis on the development of integrated pest management strategies that include the use of disease-resistant strawberry varieties, crop rotation, soil health improvement, and the integration of both biological and chemical control methods (Triasih et al., 2023; Zavatta et al., 2021; Li et al., 2022; Tane, 2022). Another measure employed to limit *Fusarium* spp. attack includes the application of fungicides or hydrogen peroxide before planting the stolons (Dara et al., 2020). Both fungicides and hydrogen peroxide can effectively reduce the population of *Fusarium* spp. in the soil or on the plant material, decreasing the initial inoculum and lowering the risk of infection (Audenaert et al., 2010). Hydrogen peroxide, a more environmentally friendly option compared to synthetic fungicides, offers a way to manage *Fusarium* spp. (La Placa et al., 2022) without contributing to chemical residue (Ng et al., 2021).

This paper seeks to illustrate how hydrogen peroxide in combination with azoxistrobin treatments can seamlessly be incorporated into current crop management frameworks and align with various control strategies, within the framework of an Integrated Pest Management (IPM) approach.

## MATERIALS AND METHODS

This study was conducted in a strawberry field located in Giurgiu County. Observations were made over a 35-day period during July and August of 2022 and 2023, utilizing the 'Alba' strawberry genotype. The experimental layout comprised four treatment variants (Table 1), with each variant assigned to a separate matted row for consistency in application and observation.

In the V2 and V4 variants, a 35% hydrogen peroxide solution was utilized for the pre-plant treatment of strawberry stolons. Prior to planting, strawberry stolons were carefully selected to ensure uniformity in size and health status. These stolons were then submerged in the 35% hydrogen peroxide solution for a duration of 30 minutes. This immersion period was determined to maximize pathogen suppression while preventing phytotoxicity.

Table 1. Treatments used to control *Fusarium* spp. in the trials

| Variant | Active ingredient | Rate (l/ha; %) |
|---|---|---|
| V1 – Control | - | - |
| V2 - pre-planting immersion of strawberry stolons in hydrogen peroxide solution and drip application of azoxystrobin during the vegetation period | Azoxistrobin | 1 |
| | Hydrogen peroxide | 35 |
| V3 - drip application of azoxystrobin during the vegetation period | Azoxistrobin | 1 |
| V4 - pre-planting immersion of strawberry stolons in hydrogen peroxide solution | Hydrogen peroxide | 35 |

Following immersion, stolons were rinsed with clean water to remove any residual hydrogen peroxide and immediately planted in the prepared field plots. In V2 and V3 variants, azoxystrobin, a broad-spectrum fungicide, was selected for its efficacy against *Fusarium* spp. The concentration of azoxystrobin used for drip application were 1l/ha. A drip irrigation system was installed in the field to facilitate precise and targeted application of the azoxystrobin solution directly to the root zone of the strawberry plants. The system comprised drip lines placed along each strawberry row, ensuring even distribution of the treatment solution. The azoxystrobin treatment was applied three times during the vegetation period. The initial application was timed to coincide with the onset of the first visible symptoms of *Fusarium* spp. infection (Figure 1), typically observed 10 days post-planting of the untreated control. Subsequent applications followed at 7-day intervals to maintain effective fungicide levels in the soil and to suppress the pathogen's development.

Figure 1. Symptoms of *Fusarium* spp. on strawberry

Observations on the frequency and intensity of *Fusarium* spp. attacks were conducted before and after each treatment, involving the visual assessment of 500 plants per variant.

The efficacy of the treated variants was evaluated against the untreated control variant using a rating scale from 0 to 100%. Efficacy calculations for the three treatment variants followed Abbott's formula:

$$E(\%) = \frac{DA\ \ in\ the\ control - DA\ in\ the\ treated\ variant}{DA\ \ in\ the\ control} \text{ x } 100$$

**Diagnosis and confirmation of *Fusarium* spp.** Symptomatic strawberry plants displaying signs of wilting, yellowing, and crown discoloration were systematically collected from the field. Preliminary diagnosis involved detailed visual inspection of these samples to assess characteristic *Fusarium* wilt symptoms. For the isolation of *Fusarium* spp., segments from symptomatic roots and crowns were surface sterilized and plated on PDA medium. Plates were incubated at approximately 25°C, allowing for fungal growth observation over 5-7 days. Morphological identification was conducted on the presumptive *Fusarium* colonies, examining specific fungal structures under a microscope (Figure 2). Healthy strawberry plants were test for *Fusarium* spp. as the cause of wilt, following Koch's postulates. Strawberry seedlings, specifically 'Alba' genotype, were transplanted into sterilized soil inoculated with a fungal conidial suspension (10^6 spores/ml). The seedlings were monitored for wilt symptoms and re-isolated when symptoms appeared. This allowed a comparison between the morphological characteristics of the re-isolated *Fusarium* spp. and the initial inoculum, confirming Koch's postulates and linking *Fusarium* spp. infection to the wilt symptoms in the strawberry crops.

Figure 2. Mycelial growth and fungal structures of *Fusarium* spp.

**Statistical analysis.** Collected data were statistically analysed by ARM-9 software using ANOVA test analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) tests.

## RESULTS AND DISCUSSIONS

Following the observations made over the two years, specifically 2022-2023, it was noted that *Fusarium* spp. infections occurred during the summer planting period in July-August and had devastating effects on untreated plants, with the infection spreading very quickly (Table 2).

Table 2. Frequency and intensity of *Fusarium* spp.

| Observation days | Assesment | Control (V1) | V2 | V3 | V4 |
|---|---|---|---|---|---|
| Before treatment | Frequency | 47.35a | 19.03d | 24.68c | 22.95b |
| | Intensity | 100a | 45.58d | 59.70b | 50.70c |
| First treatment | Frequency | 53.63a | 18.21d | 28.27b | 22.83c |
| | Intensity | 100a | 45.46d | 60.86b | 50.26c |
| 7 days after first treatment | Frequency | 56.07a | 16.20d | 32.93b | 21.08c |
| | Intensity | 100a | 46.50b | 50.23b | 49.88b |
| Second treatment | Frequency | 59.78a | 15.37d | 33.56b | 20.04c |
| | Intensity | 100a | 37.19d | 65.47b | 49.55c |
| 7 days after second treatment | Frequency | 61.10a | 15.08d | 37.76b | 19.45c |
| | Intensity | 100a | 34.12d | 66.00b | 47.69c |
| Third treatment | Frequency | 66.61a | 14.00d | 42.45b | 17.67c |
| | Intensity | 100a | 34.63d | 68.66b | 44.66c |
| 7 days after third treatment | Frequency | 68.66a | 12.64d | 43.30b | 17.78c |
| | Intensity | 100a | 33.96d | 70.08b | 44.28c |
| 14 days after third treatment | Frequency | 70.24a | 10.30d | 44.11b | 15.28c |
| | Intensity | 100a | 28.68d | 70.16b | 40.15c |
| LSD P = .05 | | 16.007 | 12.594 | 14.158 | 15.585 |
| Standard deviation | | 21.15 | 12.91 | 15.97 | 14.5 |

The letters (a, b, c, d) next to the numerical values indicates statistical significance in a descending order where 'a' is the highest or most severe and 'd' is the lowest or least severe, according to Tukey's HSD test; P< 0.05.

According to the data presented in Figure 3, while the severity of the attack increased in the control group, a noticeable decrease was observed in all other experimental groups.

The climatic conditions during this period were characterized by high daytime temperatures 27-35°C, typical for this time of year. Precipitation was almost nonexistent, and the necessary moisture was provided through the use of a drip irrigation system.

Facing a rise in *Fusarium* spp. attacks on strawberries, our research shifted from standard treatments to a higher hydrogen peroxide concentration (35%).

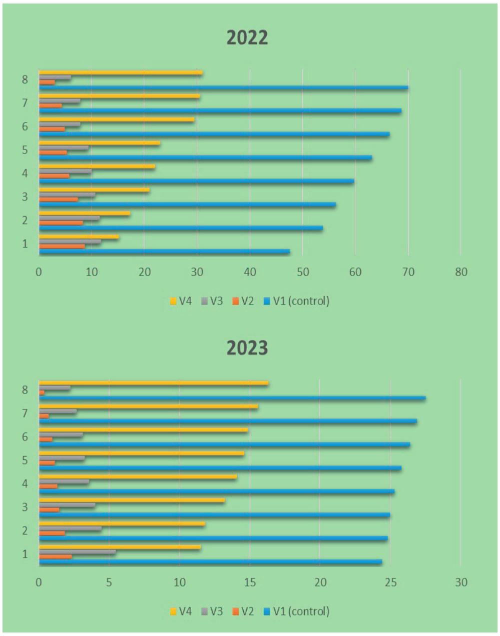


Figure 3. DA% of *Fusarium* spp. in strawberry crop 2022/2023

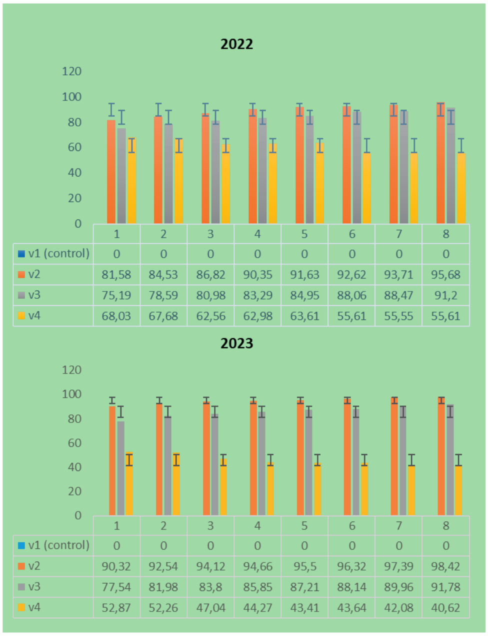


Figure 4. Efficacy of hydrogen peroxide and azoxistrobin treatments in the attack of *Fusarium* spp. in strawberry crop 2022/2023

This was based on the idea that a stronger dose might significantly disrupt the pathogen, preventing its spread without harming the strawberry plants. Observations confirmed that this approach did not adversely affect the plants while potentially offering a new, effective method to combat *Fusarium* spp. In Romania, numerous studies have been conducted concerning the calculation of treatment efficacy, particularly in research focused on the management of plant diseases (Buzatu et al., 2018; Toth et al., 2020; Jalobă et al., 2019; Alexandru et al., 2019; Iosub et al., 2022). The best results of efficay were observed in Variant 2 both in 2022 and 2023 (Figure 3), where the immersion of roots in a hydrogen peroxide solution stalled the development of the pathogen, and azoxystrobin reduced the number of infections. High efficacy in limiting *Fusarium* attack was also observed in Variant 3, which consisted of the drip application of azoxystrobin. A reduction in pathogen spread was noted in Variant 4 as well, but the efficacy decreased once the antifungal effect of the 35% hydrogen peroxide solution was no longer present on the strawberry plant roots. The untreated control showed a rapid upward trend due to the absence of treatments.

The analysis of variance (ANOVA) revealed a significant difference between the treatment groups ($p < 0.05$), in 2022, indicating that at least two treatment variants had significantly different effects on controlling *Fusarium* spp. infections. Post-hoc Tukey's Honestly Significant Difference (HSD) tests were conducted to determine specific pairwise differences between treatment means. The results showed significant differences between Control (V1) and each of the treatment variants (V2, V3, and V4) ($p < 0.05$), indicating that all three treatment variants were more effective in controlling *Fusarium* spp. infections compared to the control group. Additionally, significant differences were observed between V2 and V4 ($p < 0.05$), as well as between V3 and V4 ($p < 0.05$), suggesting that the combined treatment involving hydrogen peroxide and azoxystrobin (V2) and the treatment with azoxystrobin alone (V3) were more effective than the treatment with hydrogen peroxide alone (V4). However, no significant difference was found between V2 and V3 ($p > 0.05$), indicating comparable effectiveness between the two treatments involving azoxystrobin. Also, in 2023, V2 exhibited significantly higher efficacy compared to the control group (V1) and other treatment variants (V3 and V4). Additionally, significant differences were observed between V2 and V4, as well as between V3 and V4, further highlighting the superior efficacy of V2. Overall, the results of the 2022/2023 study (Table 3) indicate that treatment variant V2 involving hydrogen peroxide and azoxystrobin stands out as the most effective strategy for managing *Fusarium* spp. infestations in strawberry crops.

Table 3. Comparative analysis of treatment variants for *Fusarium* spp. control in strawberry crop 2022/2023

| Comparison | Year | Mean Difference | Standard Error (HSD) | Standard Deviation | p-value | Statistically Significant |
|---|---|---|---|---|---|---|
| Control (V1) vs. V2 | 2022 | 89.615 | 6.665 | 0 | 0.0000000 | Yes |
| Control (V1) vs. V3 | 2022 | 8.384.125 | 6.665 | 0 | 0.0000000 | Yes |
| Control (V1) vs. V4 | 2022 | 6.145.375 | 6.665 | 0 | 0.0000000 | Yes |
| V2 vs. V3 | 2022 | 577.375 | 6.665 | 5.419 | - | No |
| V2 vs. V4 | 2022 | 2.816.125 | 6.665 | 5.260 | - | Yes |
| V3 vs. V4 | 2022 | 223.875 | 6.665 | 4.861 | - | Yes |
| Control (V1) vs. V2 | 2023 | 9.490.875 | 5.174 | 0 | 0.0000000 | Yes |
| Control (V1) vs. V3 | 2023 | 857.825 | 5.174 | 0 | 0.0000000 | Yes |
| Control (V1) vs. V4 | 2023 | 4.577.375 | 5.174 | 0 | 0.0000000 | Yes |
| V2 vs. V3 | 2023 | 912.625 | 5.174 | 2.622 | - | Yes |
| V2 vs. V4 | 2023 | 49.135 | 5.174 | 2.622 | - | Yes |
| V3 vs. V4 | 2023 | 3.900.875 | 5.174 | 4.587 | - | Yes |

These findings suggest that the combined treatment of hydrogen peroxide and azoxystrobin (V2) and the treatment with azoxystrobin alone (V3) exhibit promising efficacy in controlling *Fusarium* spp. infections in strawberry crops, warranting further investigation and potential adoption in agricultural practices.

Studies have shown that applying hydrogen peroxide to transplanted seedlings improves their development and growth. Furthermore, changes were noted in the soil microhabitat, most notably in the relative abundance of *Bacillus* and *Mortierella*, which were both on the rise, and *Fusarium*, which was on the decline. Replanted soil treated with 4.5% hydrogen peroxide was shown to be the most effective therapy overall, greatly reducing the impacts of apple replant disease (ARD) (Xu et al., 2023). Oberländer et al. (2018), sugest that hydrogen peroxide possesses microbicidal and sporicidal activity and the addition of different concentrations of hydrogen peroxide can significantly reduced the bacteria, fungi, and *Fusarium oxysporum* numbers in the soil.

Previous investigations into the use of azoxystrobin as a fungicidal agent against *Fusarium* spp. have yielded mixed results. While some studies report limited inhibitory effects (Akram et al., 2018; Pirgozliev et al., 2002) of azoxystrobin on the pathogen, others have highlighted its potential to significantly inhibit *Fusarium* spp. growth under certain conditions (Degani et al., 2021; Song et al., 2022).

This variation in reported efficacy underscores the complexity of the pathogen-fungicide interaction and suggests that the outcome of azoxystrobin application may be influenced by a multitude of factors, including the mode of application, environmental conditions, and specific *Fusarium* species or strains targeted. The diversity in findings emphasizes the need for a nuanced understanding of how azoxystrobin performs across different agricultural contexts and suggests that optimization of application strategies could be key to enhancing its fungicidal effectiveness against *Fusarium* spp. Notably, the targeted delivery of azoxystrobin via drip irrigation in our experiment ensured more direct and efficient fungicide contact with the root zone, potentially enhancing its efficacy. Furthermore, the specific environmental conditions of Giurgiu County during the study period, along with the possible variation in *Fusarium* strain susceptibility to azoxystrobin, might have contributed to the observed increased effectiveness. Our findings suggest that the efficacy of azoxystrobin in *Fusarium* suppression can significantly vary based on application techniques and context-specific factors, underscoring the importance of tailored pest management strategies in agricultural practices.

## CONCLUSIONS

The successful deployment of a 35% hydrogen peroxide solution, which demonstrates efficacy in inhibiting *Fusarium* pathogen development without harming strawberry plants, represents a notable advancement in the quest for potent, yet crop-safe, antifungal treatments. This strategy, born from the ongoing battle against *Fusarium* infections in strawberry culture, underscores the critical need for inventive and courageous approaches within the realm of agricultural pest and disease control. Consequently, the practice of pre-planting immersion of strawberry stolons in a 35% hydrogen peroxide solution, supplemented with azoxystrobin applications during the growing season, emerges as a promising alternative. This method not only diminishes *Fusarium* infestations but also reduces reliance on conventional chemical fungicides, highlighting a path towards more sustainable and environmentally conscious farming practices.

## REFERENCES


Alexandru, I., Cristea, S., & Hoza, D. (2019). Effectiveness of treatments on the attack of *Polystigma rubrum* pathogens and *Stigmina carpophila* on plum in Șoimari location, Prahova County.

Akram, S., Khan, S. M., Khan, M. F., Khan, H. U., Tariq, A., Umar, U. U., & Gill, A. (2018). Antifungal activity of different systemic fungicides against *Fusarium oxysporum* f. sp. *lycopersici* associated with tomato wilt and emergence of resistance in the pathogen. *Pakistan Journal of Phytopathology*, *30*(2), 169-176.

Audenaert, K., Callewaert, E., Höfte, M., De Saeger, S., & Haesaert, G. (2010). Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. *Bmc Microbiology*, *10*, 1-14.

Buzatu, M. A., Costache, M., Hoza, D., Șovărel, G., & Cristea, S. (2018). The efficacy of different treatments for pathogens control on the eggplant crops in the field.

Dara, S. K. (2020). Impact of drip application of fungicides on strawberry health and yields. UCANR *eJournal of Entomology and Biologicals*.

Degani, O., & Kalman, B. (2021). Assessment of commercial fungicides against Onion (Allium cepa) basal rot disease caused by *Fusarium oxysporum* f. sp. *cepae* and *Fusarium acutatum*. *Journal of Fungi*, *7*(3), 235.

FAO. (2021). Crops and Livestock Products: Strawberries [https://www.fao.org/faostat/en/#data/QCL] FAOSTAT.

https://www.reportlinker.com/clp/country/2878/726388

Iosub, L. M., Radu, M., Mihalaşcu, C., & Cristea, S. (2022). The effectiveness of treatments in the control of wheat diseases, Moara Domnească location, Ilfov County.

Jalobă, D., Jinga, V., & Cristea, S. (2019). Research on effectiveness of some fungicides treatments on Jonathan apple variety for apple scab control in Voineşti Area.

Joshi, R. (2018). A review of Fusarium oxysporum on its plant interaction and industrial use. *J. Med. Plants Stud*, *6*(3), 112-115.

La Placa, L., Giorni, P., Mondani, L., Magan, N., & Battilani, P. (2022). Comparison of Different Physical Methods and Preservatives for Control of Fusarium proliferatum Rot in Garlic. *Horticulturae*, *8*(12), 1203.

Li, Q., Zhang, D., Song, Z., Ren, L., Jin, X., Fang, W., ... & Cao, A. (2022). Organic fertilizer activates soil beneficial microorganisms to promote strawberry growth and soil health after fumigation. *Environmental Pollution*, *295*, 118653.

Ng, C. A., Poštulková, M., Matoulková, D., Psota, V., Hartman, I., & Branyik, T. (2021). Methods for suppressing Fusarium infection during malting and their effect on malt quality.

Oberländer, J., Mayer, M., Greeff, A., Keusgen, M., Schöning, M. J. (2018). Spore-based biosensor to monitor the microbicidal efficacy of gaseous hydrogen peroxide sterilization processes. *Biosens. Bioelectron*, 104, 87−94.

Pastrana, A. M., Borrero, C., Pérez, A. G., & Avilés, M. (2023). Soilborne pathogens affect strawberry fruit flavor and quality. *Plant Science*, *326*, 111533.

Pirgozliev, S. R., Edwards, S. G., Hare, M. C., & Jenkinson, P. (2002). Effect of dose rate of azoxystrobin and metconazole on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in wheat grain. *European Journal of Plant Pathology*, *108*, 469-478.

Song, Y., Chen, X., Sun, J., Bai, Y., Jin, L., Lin, Y., ... & Chen, Y. (2022). *In vitro* determination of sensitivity of *Fusarium fujikuroi* to fungicide azoxystrobin and investigation of resistance mechanism. *Journal of Agricultural and Food Chemistry*, *70*(31), 9760-9768.

Toth, K., & Cristea, S. (2020). Efficacy of treatments in controlling cercosporiosis (*Cercospora beticola* Sacc.) in sugar beet.

Triasih, U., Harsonowati, W., Dwiastuti, M. E., & Widyaningsih, S. (2023, December). Characterization of Fusarium Wilt Disease Among Different Strawberry (Fragaria sp.) Cultivars In Indonesia. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1287, No. 1, p. 012022). IOP Publishing.

Tane, M. C. (2022). The main fungal diseases in strawberries crop-review.

Warner, R., Wu, B. S., MacPherson, S., & Lefsrud, M. (2021). A review of strawberry photobiology and fruit flavonoids in controlled environments. *Frontiers in Plant Science*, *12*, 611893.

Xu, X., Zhou, Y., Wang, X., Jiang, W., Qin, L., Wang, J., ... & Mao, Z. (2023). Effect of Hydrogen Peroxide on the Soil Microbial Community Structure and Growth of *Malus hupehensis* Rehd. Seedlings under Replant Conditions. *ACS omega*, *8*(7), 6411-6422.

Yang, H., Zhang, X., Qiu, X., Chen, J., Wang, Y., Zhang, G., ... & Yan, Z. (2023). Fusarium Wilt Invasion Results in a Strong Impact on Strawberry Microbiomes. *Plants*, *12*(24), 4153.

Zavatta, M., Muramoto, J., Mazzola, M., & Shennan, C. (2021, May). Rotation length, crop rotation, anaerobic soil disinfestation and mustard seed meal affect organic strawberry yield and soil-borne disease incidence in California. In *IX International Strawberry Symposium 1309* (pp. 501-508).