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FURTHER STUDIES ON THE DAMAGES ON *FRAGARIA ANANASSA* VAR. 'SNOW WHITE' CAUSED BY *ERWINIA AMYLOVORA* AND APPLICATION OF CHAENOMELES PLANT EXTRACT FOR REDUCTION OF SYMPTOMS

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Abstract

Erwinia amylovora causes the well-known disease "fire blight", which affects a large number of plants from the Rosaceae family. Strawberries are also among the pathogen's hosts. The present study aimed to further investigate the damages that E. amylovora induces in greenhouse-grown strawberries (Fragaria ananassa) var. "Snow White" in Bulgaria and the potential of a plant-derived extract from fruits of Chaenomeles sp. to reduce the symptoms for another vegetation period following early infection. The experiment includes four irrigation and fertilization levels, as well as a comparison between the symptoms of the disease in the greenhouse and those induced in optimal laboratory conditions. The results showed greater leaf and flower losses in the diseased plants compared to the healthy plants. Treatments with the plant extract reduced the manifestation of symptoms below the levels observed in the diseased plants.

Key words*: Erwinia amylovora, Fragaria ananassa, disease control, Chaenomeles sp., plant extract.*

INTRODUCTION

Erwinia amylovora is a pathogen affecting numerous crops and other plants belonging to the family Rosaceae, thus posing a serious challenge for agricultural producers. The pathogen is difficult to control, and copper-based products are still extensively used, resulting in their accumulation in the environment. In research for safer alternatives, scientists have investigated the potential of botanical extracts as sources of antimicrobial compounds. Although such studies have been extensively conducted over the years, most have focused on applications in medicine, pharmaceuticals, food technology, and cosmetology, whereas investigations in plant pathology remain scarce (Jantova et al., 2000; Borges et al., 2018; Gutiérrez-Pacheco et al., 2019). Only a limited number of plant-derived products have been examined for their activity against plant pathogenic bacteria (Stangarlin et al., 1999; Schwan-Estrada et al., 2005; Mohamed, 2010; Bhagwat & Datar, 2014; Stoyanova & Valkova, 2014, 2015a, 2015b; Ganiyu et al., 2017). This is largely due to the enormous diversity of plant species as well as the wide range of phytopathogenic microorganisms. Additional factors, including strain differences, extraction procedures, application methods, and host specificities, further contribute to the global pace of research and the still limited availability of data.

Several plant extracts demonstrated antibacterial activity against *E. amylovora in vitro* (Mosch et al., 1990; Mosch & Zeller, 1996; Chiriac & Ulea, 2012; Stoyanova & Valkova, 2014; Fontana et al., 2022; Stoyanova et al., 2024). A recent *in vivo* study revealed a promising potential of a methanol extract from *Chaenomeles* sp. fruits to control the pathogen when applied as preventive sprays under greenhouse conditions (Stoyanova et al., 2025). Chaenomeles is widely distributed throughout Asia and Europe and exhibits considerable genetic diversity. Nevertheless, current evidence suggests that the antibacterial activity against *E. amylovora* is not restricted to certain genotypes (Stoyanova et al., 2024).

The aim of the present study was to further investigate the damages induced by *E. amylovora* in greenhouse-grown strawberries (*Fragaria ananassa*) var. "Snow White" in Bulgaria and the potential of a plant-derived extract from fruits of *Chaenomeles* sp. to reduce the symptoms during the next vegetative period under conditions of early infection.

MATERIALS AND METHODS

Plant extract. The extract was derived from frozen fruits of *Chaenomeles* sp., genotype 29, originating from the field of the Research Institute of Mountain Stockbreeding and Agriculture, Agricultural Academy, Troyan, by Soxhlet extraction in methanol (HPLC grade) at 80°C for 5 hours. The solvent was recovered in a vacuum vaporizer (model Hei VAP Precision, Heidolph Inst.) at 55°C, 30 000 Pa, and the extracts were concentrated at decreasing pressure down to 7 200 Pa and a temperature of 65°C. The extract was stored at 18°C, in the dark until use and applied as a freshly prepared 2% (w/v) aqueous solution by spraying, based on previous studies (Stoyanova et al., 2024, 2025).

Bacterial strain. The test strain *Erwinia amylovora* NBIMCC 2331 (=ATCC 15580, type strain, isolated from pear) was applied by spraying as water suspensions with 75% transmittance (measured with a turbidimeter Biolog Inc.) for plant inoculation.

Vacuum infiltration. Young plants were inoculated following the method of Jones et al. (1981). Plants were immersed in a bacterial suspension at 85% transmittance and subjected to vacuum in a desiccator generated by a water aspirator with a flow rate of 10 L/min, two consecutive 3-minute exposures separated by a 5-minute interval, in five replicates. Control plants were infiltrated with sterile distilled water. The experiment was performed twice, using plants from varieties "Murano" and "Snow White" in 2024 and 2025, respectively.

Plants. A total of 160 strawberry plants *Fragaria ananassa* variety "Snow White" were used for the main study. The plants were grown in a non-heated tunnel greenhouse, covered with a 150 μm five-layer UV+EVA+IR+AD+dif polyethylene film, and located at the Experimental Base "Chelopechene" of the Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Pushkarov", Agricultural Academy, Sofia, Bulgaria (42°44′22.8″N, 23°28′3.7″E). The plants were mulched with 30 μm silver-black UV polyethylene film and cultivated using drip irrigation and fertilization.

Variants of Irrigation and Fertilization (VIF). We used four VIFs: 100% irrigation (I0F0), 75% irrigation and 100% fertilization (I1F1), 75% irrigation and 75% fertilization (I1F2), 50% irrigation and 75% fertilization (I2F2).

Experimental design. For each VIF, the plants were divided into four *groups*: healthy (negative control, *H*), treated with extract (second negative control, *T*), experimental (treated with extract and inoculated with pathogen, *E*) and infected (inoculated with pathogen, *I*). Each group included 10±1 plants. The withered leaves and flowers of each plant were recorded weekly and removed from the plants prior to treatment to avoid recounting. Treatments were applied once per week for eight weeks during the period April-June 2025. Inoculations were conducted in the first and fourth weeks, one hour after treatment, to compensate for the reduced disease pressure due to the manual removal of diseased plant parts.

Statistics. The data were statistically analyzed by Duncan's Multiple Range test at significance levels of $p<0.05$ using STATISTICA 8.0 software.

RESULTS AND DISCUSSIONS

The progression of disease symptoms following infection with *E. amylovora* was monitored after vacuum infiltration of strawberry seedlings. The objective was to compare symptom development under optimal conditions for pathogen growth with the symptoms induced on infected white strawberry plants under greenhouse conditions.

Seedlings subjected to vacuum infiltration predominantly developed marginal or diffuse necrosis of the leaves, which rapidly spread across the entire leaf surface, accompanied by necrosis of the petioles. In a smaller proportion of leaves, necrotic lesions were associated with transient chlorosis. The young plants became completely desiccated within approximately three weeks. Both strawberry varieties exhibited similar symptoms, severity, and progression

rates. Similar symptom patterns were observed in the main greenhouse experiment.

Infection of leaves in the greenhouse most commonly began as marginal necrosis, which subsequently progressed to involve the entire leaf petiole, with the young leaves being more susceptible. Less frequently, diffuse necrosis could also be observed. Preceding chlorosis was observed only rarely. Symptoms on flowers included necrosis and chlorosis on sepals (Figure 1). Necrosis of the petioles or peduncles could also develop. At the final stage of infection, complete withering of the infected leaves and flowers was observed regardless of the inoculation method, with the affected organs remaining attached to the plants.

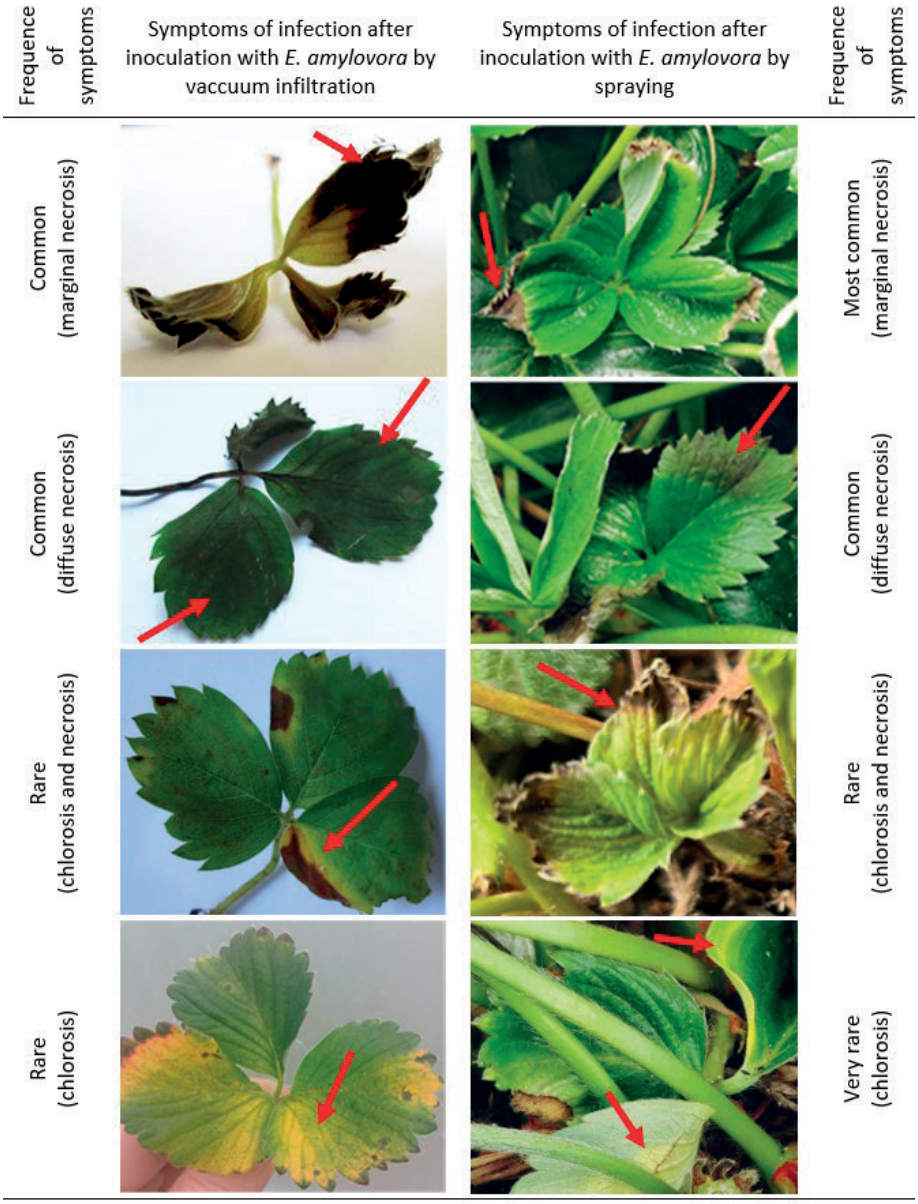

Figure 1. Symptoms of infection with *E. amylovora* on leaves and flowers of strawberry plants using two methods of inoculation - vacuum infiltration in laboratory conditions and spraying in greenhouse conditions

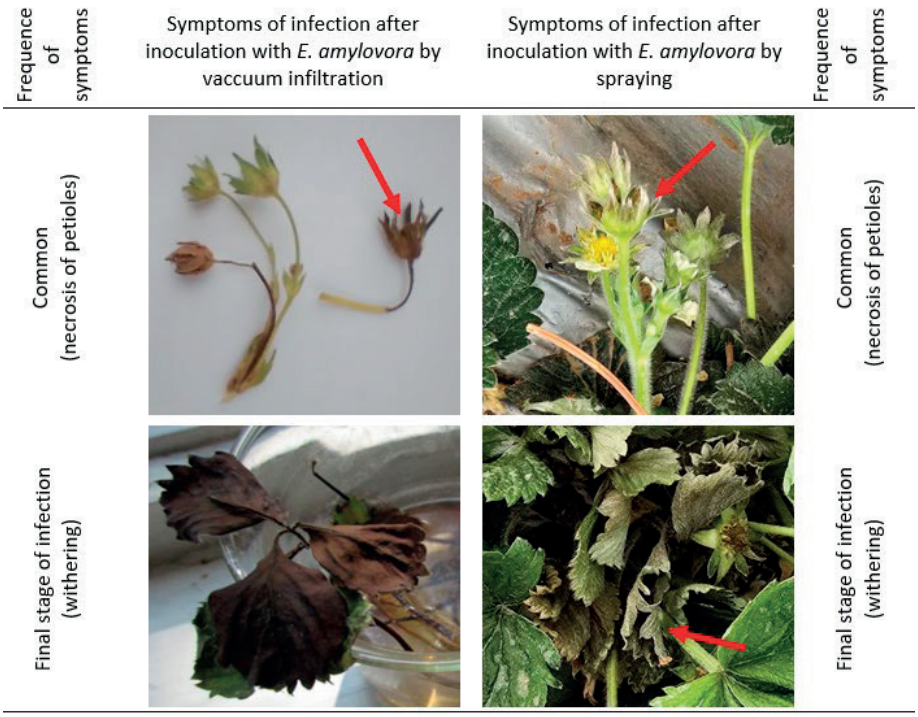

Figure 1. Symptoms of infection with *E. amylovora* on leaves and flowers of strawberry plants using two methods of inoculation - vacuum infiltration in laboratory conditions and spraying in greenhouse conditions (*continued*)

The types of symptoms of infection in greenhouse conditions during 2025 were observed at approximately the same frequency like in 2024.

In the main greenhouse experiment, infected, non-treated plants (positive control) exhibited the greatest loss of leaves and flowers compared to all other groups. The number of withered leaves in the positive control plants was 2.7-fold higher than in the negative control (non-inoculated, non-treated plants), which primarily reflects physiological senescence. Compared to treated (non-inoculated, extract-applied) and experimental (inoculated, extract-applied) plants, the positive controls displayed 3.7- and 3.6-fold greater leaf loss, respectively (normalized per plant) (Figure 2). A similar pattern was observed for flowers. Infected plants lost 14.8-, 8-, and 10.3-fold more flowers than the healthy, treated, and experimental plants, respectively (normalized per plant) (Figure 2).

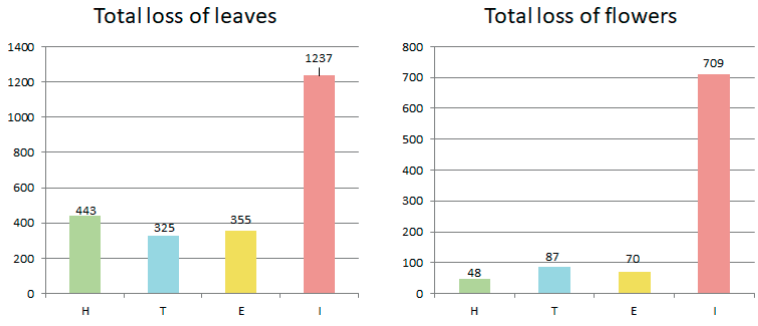

Figure 2. Loss of leaves and flowers of healthy (H), treated (T), experimental (E) and infected (I) plants for a period of eight weeks

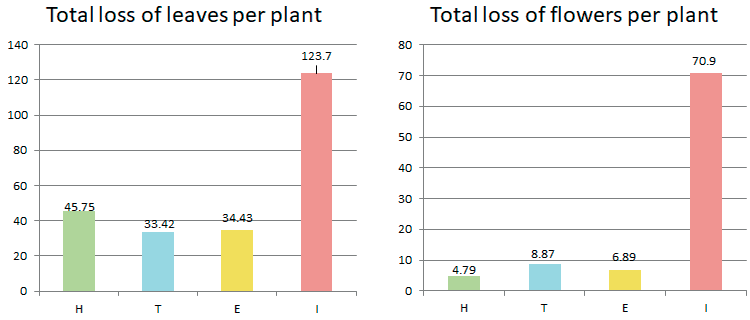

Figure 2. Loss of leaves and flowers of healthy (H), treated (T), experimental (E) and infected (I) plants for a period of eight weeks (*continued*)

Leaf and flower losses were normalized relative to the healthy control (H), yielding approximate ratios for each treatment group. According to the data, the ratios of the infected group are substantially higher compared to the ones of the treated and experimental plants (Table 1).

Table 1. Approximate ratios of leaf and flower loss calculated towards the healthy plants (values are rounded)

H : T : E : I	
leaves	flowers
1 : 0.7: 0.8 : 2.7	1 : 1.9 : 1.4 : 14.8

Statistical analysis confirmed that the differences observed in the infected plants were significant ($p < 0.05$), while the losses of the healthy, treated and experimental groups were not statistically different (Table 2). These results further indicate that the extract had no detrimental effect on strawberry plants.

Table 2. Average loss of leaves and flowers of the groups: healthy (H), treated (T), experimental (E) and infected (I)

	H	T	E	I
leaves	5.64a	4.18a	4.3a	15.46b
flowers	0.6a	1.11a	0.86a	8.85b

Values with different lowercase letter for the same parameter were statistically different.

Analysis of the recorded data with respect of the different variants of irrigation and fertilization (VIFs) distributed the leaf and flower losses in a tendency following the global one: in every variant, the plants that lost the most leaves and flowers were the infected ones. The experimental plants suffered lower leaf loss than the healthy ones in I0F0, I1F2 and I2F2, and the loss value of I1F1 leaves was closer to the healthy plants compared to the diseased ones (Figure 3). The experimental plants suffered lower flower losses than the healthy ones in I1F2, and the flower loss values of I0F0, I1F1 and I2F2 were closer to the healthy plants compared to the diseased ones (Figure 3).

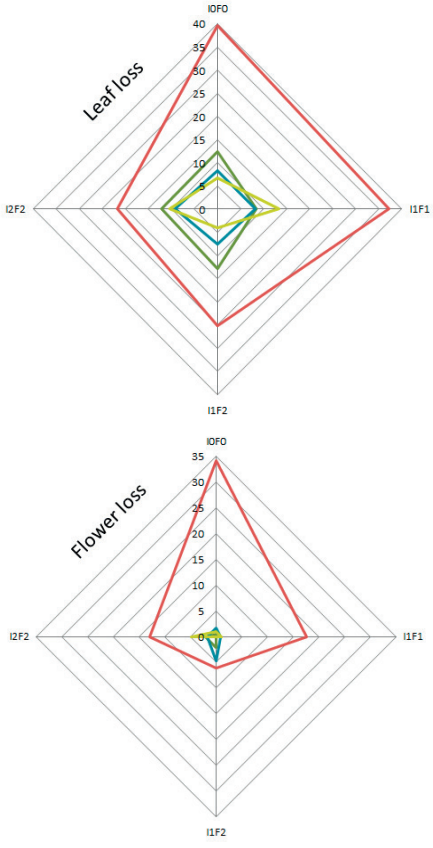

Figure 3. Leaf and flower loss per plant by VIFs

However, some differences among the groups could be observed. Bigger gaps of leaf and flower losses between the infected and the other three groups (H, T and E) were observed for variants I0F0 and I1F1. The least differences were recorded for flower loss of variant I1F2. Through the vegetation period of 2025, the lack of fertilization, in combination with optimal irrigation, favored the most the development of the disease in terms of both leaf and flower loss. When compared to the previous year, the counts of lost leaves per plant for 8 weeks are not very different as values for variants I1F1, I1F2 and I2F2, but the losses for I0F0 are much higher (Table 3). This could be explained by the continuous exhausting of the plants due to lowering of the soil nutrients.

Statistical analysis of the data on leaf loss with respect to the VIFs revealed significant differences among the groups. In all four variants, the infected plants were clearly separated from the healthy, treated, and experimental plants (Table 4).

Statistical analysis of flower loss with respect to the VIFs indicated a significant separation only between the infected plants and the I0F0. All other plants were grouped within the same statistical category, which may be attributed to the relatively low number of flowers recorded (Table 5).

Compared to the results received in 2024, the losses of leaves per plant for 8 weeks are not very different as values for three of the variants. However, the loss of flowers is significantly lower, which mainly corresponds to the significantly lower rate of flowering (Table 3). A main reason for this could be the age of the strawberry plants. Differences in temperatures could also add to these results. During 2025, the experimental period started 4 weeks earlier which was related to lower daily temperatures (Table 6). In addition, the last 4 weeks were colder compared to the same period in 2024 (Stoyanova et al., 2025) both in means of minimal and maximal values with differences up to 7 °C in the absolute values.

Table 3. Approximate ratios of leaf and flower loss of infected plants for years 2025/2024 (values are rounded)

VIFs	leaves	flowers
I0F0	1.6	0.7
I1F1	0.9	0.2
I1F2	0.8	0.1
I2F2	0.9	0.2

Values closer to 1 refer to closer count numbers for the two consecutive years.

Table 4. Average loss of leaves of healthy (H), treated (T), experimental (E) and infected (I) plants with respect to VIFs

leaves	H	T	E	I
I0F0	6.2a	4.2a	3.36a	19.85d
I1F1	4.17a	4.15a	6.7ab	18.55d
I1F2	6.09a	3.75a	2a	12.6c
I2F2	6.11a	4.61a	5.15a	10.85bc

Values with same lowercase letter for the same parameter were not statistically different.

Table 5. Average loss of flowers of healthy (H), treated (T), experimental (E) and infected (I) plants with respect to VIFs

flowers	H	T	E	I
I0F0	0.25a	0.85a	0.5a	17.05b
I1F1	0a	0.4a	0.5a	8.8a
I1F2	1.09a	2.35a	0.05a	3.05a
I2F2	1.06a	0.83a	2.4a	6.5a

Values with same lowercase letter for the same parameter were not statistically different.

Table 6. Temperature data during the experimental period (values based on 48 measurements per 24 hours, °C)

week	1st	2nd	3rd	4th	5th	6th	7th	8th
period	10.04-16.04	17.04-23.04	24.04-30.04	01.05-07.05	08.05-14.05	15.05-21.05	22.05-28.05	29.05-04.06
Absolute Min	-3.4	6.0	1.2	3.8	3.1	2.2	6.8	7.2
Daily Min	2.9±4.2	8.4±4.0	5.8±3.4	8.1±3.1	7.0±2.2	5.4±1.7	9.1±1.6	9.0±1.9
Absolute Max	35.5	42.7	40.8	43.6	39.0	39.1	41.8	46.5
Daily Max	27.8±5.3	38.4±6.0	33.7±5.5	39.4±2.9	33.2±3.8	34.2±7.9	28.5±9.3	39.2±6.5
Average	13.0±2.2	19.3±3.0	16.8±1.8	21.3±1.4	18.0±1.3	18.2±2.6	17.2±4.4	21.8±4.0

Infection with *E. amylovora* consistently induces premature leaf death in strawberries which can potentially affect plant development and physiological status. The disease also causes high flower loss, potentially affecting yield. The application of aqueous solution of the methanol extract from *Chaenomeles* sp. fruits in concentration of 2% (w/v) does not exert any negative visual effects on the plants and can significantly reduce the symptoms to levels that are characteristic to the healthy plants.

CONCLUSIONS

The study revealed that fire blight of strawberries var. "Snow White" manifests as leaf and flower loss, which may adversely affect overall plant development. The extract prepared from chaenomeles demonstrated a protective effect by limiting the development of the disease in the plants.

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