Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

ECO-FRIENDLY AND BIOLOGICAL MEANS TO REDUCE PHYTOPATOGENIC AND COLIFORM CONTAMINANTS ON STRAWBERRIES

Sorina Dinu[1], Viorel Fătu[1], Lavinia-Diana-Nicoleta Buturugă-Barbu[1,2], Oana-Alina Boiu-Sicuia[1,2,*]

[1]Research - Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., PC 013813, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnologies, 59 Mărăști Blvd., PC 011464, Bucharest, Romania

Abstract
Strawberries are sensitive to pathogens attack. Therefore, they require specific phytosanitary control measures. Antagonistic microorganisms, such as the biological control agents, have been suggested as an alternative method to prevent various infections. The aim of this work was to select beneficial yeasts and bacterial strains with inhibitory potential against the phytopathogenic fungus Botrytis cinerea, that causes gray mold. Certain in vitro tests were performed to identify the biocontrol mechanisms of action. Moreover, the presence of coliform bacteria was also evaluated on the strawberry fruits grown in organic system. Two yeast strains, Saccharomyces cerevisiae L30b and Metschnikowia pulcherrima sa5, and other six bacterial strains, Bacillus subtilis A4, Pseudomonas chlororaphis RG, P. fluorescens Rcp2, R2.1d and 79.3 and Serratia plymuthica Ps33, were tested in vitro for the production of diffusible and volatile compounds with antifungal effect. The results highlighted Pseudomonas fluorescens R2.1d strain which had the best efficacy on inhibiting the pathogenic mycelium growth. This strain released both diffusible and volatile antagonistic compounds against gray mold caused by B. cinerea, with as efficacy of 68.9% and 88.9%, respectively. The strawberries surface microbiota was evaluated on fruits collected from an ecological production system, having different technological option as experimental variants. The lowest microbial load of coliform bacilli was shown on the strawberry fruits collected from the matted row variant, covered with black foil mulch, compared to the other technological option of the ecological production system.

Keywords: antagonistic strains, biocontrol, Botrytis cinerea, strawberries, total coliforms.

1. INTRODUCTION

Strawberries are sensitive to the attack of pathogens therefore, require specific phytosanitary control measures (Maas, 1998; Agrios, 2005). Antagonistic microorganisms, such as biological control agents (BCAs), have been suggested as an alternative method to prevent various infections in strawberries (Magnin-Robert et al., 2013; Spadaro and Droby, 2016). Microorganisms often used as biocontrol agents are bacteria, yeasts and filamentous fungi. Several mechanisms of action are known to be involved in the biological control of phytopathogens (Huang et al., 2011; Zhang et al., 2007; Compant et al., 2005). However, using compatible BCAs, with complementary biological effects, a higher protection level against phytopathogenic attack can be triggered.

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

BCAs are able to compete pathogenic microorganisms, reduce their growth by antibiosis, hyperparasitism, or by producing various antimicrobial compounds, enzymes, as well as volatile organic compounds (VOCs). Moreover, BCAs can induce plant resistance against phytopathogenic attack, or strengthen host plant's defense against infections (Mangunwardoyo et al., 2011). In recent years, many studies have been carried out for the isolation and characterization of antagonistic microorganisms against strawberry pathogens.
The aim of this was to select beneficial BCAs strains with inhibitory potential against *Botrytis cinerea* phytopathogenic fungus that causes gray rot, and to identify the antifungal mechanisms of action, through different in vitro tests. Evaluation of total microbial load and coliforms present on strawberry fruits, grown in organic system, was another objective of this study.

2. MATERIALS AND METHODS

Antagonistic strains.

The autochthonous yeasts and bacterial strains used in this study belonging to the RDIPP and UASVM Bucharest microbial collections. There strains were isolated from various sources (table 1), and selected due to their antagonistic potential, proven against different fungal phytopathogens.

Table 1. The strains of antagonistic microorganisms used in in vitro tests

Beneficial microorganism	Strain code	Isolation source	Provenance	Notes
Saccharomyces cerevisiae	L30b	grape berry	RDIPP	DSMZ 23648; patent no. 127523/2015 (Oancea et al., 2015a)
Metschnikowia pulcherrima	sa5	grape berry	UASVM Bucharest	Classical microbiological identification (Boiu-Sicuia et al., 2020)
Serratia plymuthica	Ps33	wheat rhizosphere	RDIPP	16S rRNA gene sequencing identification; NCBI GenBank, EU1181134; patent no. 127469/2015 (Oancea et al., 2015b)
Pseudomonas chlororaphis	RG	wheat rhizosphere	RDIPP	BIOLOG identification
P. fluorescens	79.3	soil	RDIPP	BIOLOG identification
P. fluorescens	Rap2.1d	rapeseed soil	RDIPP	BIOLOG identification
P. fluorescens	Rcp2	onion rhizosphere	RDIPP	BIOLOG identification
Bacillus subtilis	A4	wheat rhizosphere	RDIPP	BIOLOG identification

Fungal pathogen. Phytopathogenic fungus *Botrytis cinerea* was used. This pathogen was isolated from rotten strawberry fruits, collected from plants grown in RDIPP experimental field (figure 1).

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

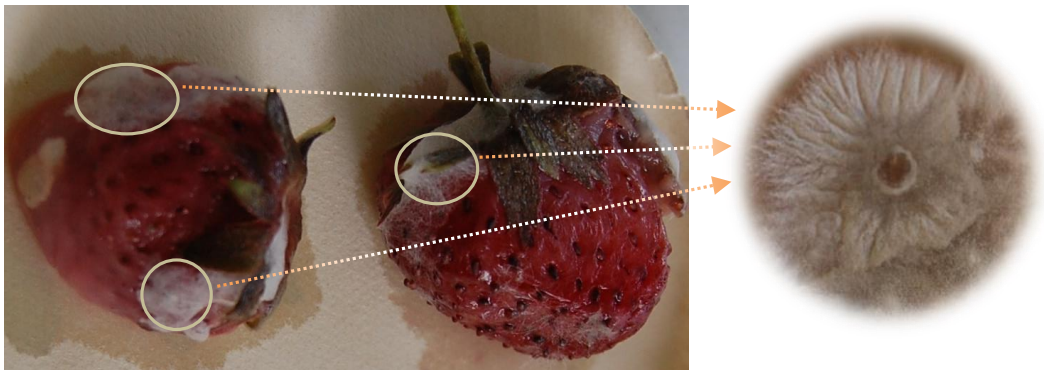


Figure 1. Rotten strawberry fruits naturally contaminated with gray mold and B. cinerea on YPD agar (photo: S. Dinu).

All microorganisms were grown on agar YPD medium (1% yeast extract, 2% peptone, 2% D-dextrose, 2% agar-agar, distilled sterile water up to 1000mL), incubated at optimal temperatures for each species respectively, 28°C for antagonistic strains and 25°C for *Botrytis cinerea*.

In vitro antagonistic activity

To evaluate the interactions between the antagonistic strains and *B. cinerea*, both types of microorganisms were co-cultivated on YPD agar plate, in three replicates. The phytopathogenic fungus was calibrated as mycelium discs, 5×5 mm^2, collected from a young culture. Each petri plate was inoculated with both types of microorganisms, antagonistic yeast or bacteria towards fungal phytopathogen, and subsequently sealed with parafilm. Simultaneously, a control plate was prepared, with only the phytopathogenic fungus. The inoculated plates were kept in the dark and incubated at 25°C for 5 days. After this first screening, the antagonistic microorganisms were further tested for the synthesis of diffusible and volatile antifungal substances.

Diffusible compounds analysis

Production of diffusible compounds was tested by dual culture method on YPD agar, using the modified method of Lopes et al. (2015). Each antagonistic strain was streaked near the edge of a petri dish, while a mycelium disc of *B. cinerea* was placed in the opposite side of the dish (figure 2). All experimental variants had three replicates and entire test was repeated twice.

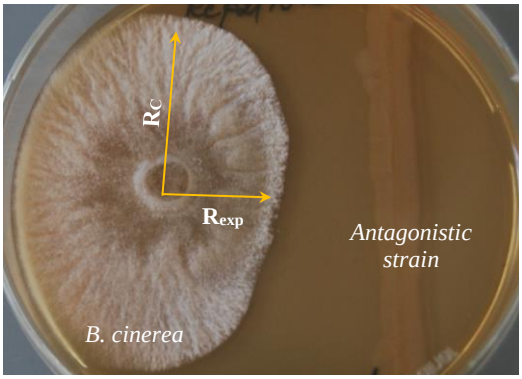


Figure 2. Metabolite diffusion assay in dual culture

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

After 5 days incubation at 25°C, the phytopathogenic mycelium growth was measured. The inhibition rate was calculated according to the following formula: [(Rc – Rexp) / Rc] × 100 which shows the inhibitory effect. The Rc is the longest diameter of fungal mycelium and Rexp is the horizontal diameter of the pathogenic mycelium.

Volatile organic compounds analysis

To evaluate the production of microbial VOCs another antagonistic assay was performed. The inverted plate method was used, while the inoculations were made on YPD agar. Both types of studied microorganisms, the antagonistic strains and the phytopathogen, were each inoculated on the bottom part of a petri dish. The plates were then assembled together, in "mouth to mouth" position, and sealed together with parafilm foil (Arrebola et al., 2010), as in figure 3. An amount of 50µl of spectrophotometrically calibrated inoculum was used form each antagonistic strain. For the bacterial strains, the inoculum was prepared at 10^8cfu/ml, while for the yeast strains, the inoculum was 10^7cfu/ml, respectively. The pathogenic mycelium was collected from 72-old culture, and calibrated as 5×5mm^2 discs.

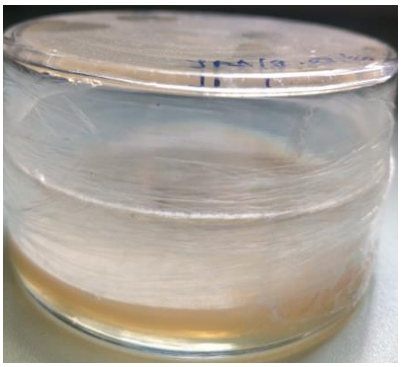


Figure 3. Volatile organic compounds assay by inverted plate method

The mycelium growth in the test and control plates was biometrically evaluated after 5 days of incubation in the dark, at 25°C. To study the efficacy of the selected strains to inhibit the growth of *B. cinerea*, the radial growth of fungal mycelium was measured in two randomly chosen directions. The inhibition rate was calculated as: [(Rc^2 – $Rexp^2$) / Rc^2] × 100, where Rexp is the fungal mycelium diameter in presence of antagonistic strain, while Rc is the diameter of fungal mycelium in control plate. Each treatment variant had three replicates and the entire experiment was repeated twice.

Determination of surface bacteria

The RIDA-STAMPS (RS) ready-made agar plates were used for quantitative detection of surface microorganisms, according to the specifications. Total microbial count and coliform bacteria present on the surface of strawberry fruits was evaluated. These fruits were collected from RDIPP experimental field where strawberries were grown in organic/ecologic system. Four technological option of organic production were present in the experimental field: a control plot, where plants were grown non-mulched flat ground (V1), an experimental plot on flat ground, covered with straw mulch layer (V2), a matted row plot without mulch (V3), and a matted row plot covered with plastic foil mulch (V4).

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

RS Total plates were used for the enumeration of total culturable aerobic, mesophilic organisms. On the RS Total plates, different colored colonies, as well as colorless and transparent colonies could be achieved. Test strains, such as *Staphylococcus aureus* ATCC 6538, *S. epidermidis* S. epi-1, *Escherichia coli* EC-68; *Citrobacter* spp. Cit-2 should produce visible growth after 20-24 hours of incubation, otherwise the incubation time should be extended to 48 hours.
The RS Coliform plates were used for the detection of coliform bacteria, which can be both quantified and differentiated based on their colony color. Other bacterial species are not able to grow on this medium or form differently colored colonies. Reference strains are used for orientation. *E. coli* ATCC8739 and *Citrobacter freundii* ATCC8090 are producing pigmented colonies, while *Pseudomonas aeruginosa* ATCC9027 is producing colorless colonies. As the coliform bacteria contain β-galactosidase enzyme capable of cleaving the chromogenic X-GAL substrate contained in agar, these bacteria are the only ones forming the typical blue/green colonies. To determine the total number of coliform bacteria inoculated plates were incubated at 36°C ±1°C. A visible growth should be observed after 18-22 hours, otherwise the incubation time is extended to 48 hours.

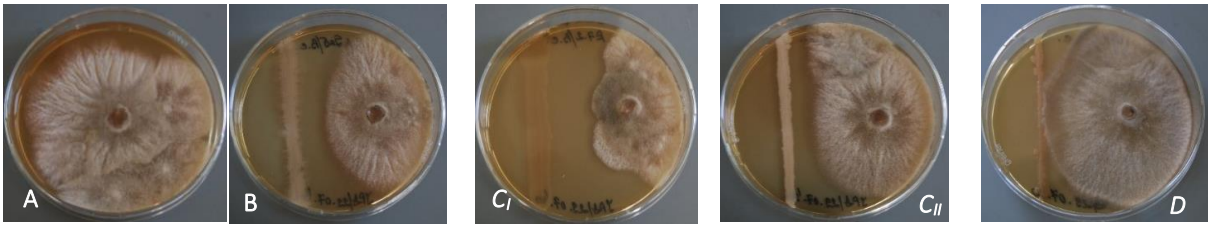
Preparation of samples.

Strawberry fruits were harvested from the experimental field where plants were grown in four technological option of organic production. A number of 15 strawberry fresh fruits were used from each experimental variant. Fruits were washed with tap water to removing the adhering soil. Without being disinfected, fruits were sectioned with a sterile scalpel in the apical part. The direct imprinting surface was flat, smooth, without any protruding compounds that could damage the agar surface. For each experimental variant, 3 plates (replicates) were made. The imprinted agar plates were incubated upside down for 24-48 h, at recommended temperature for each agar type respective, 30-35°C for RS Total plates and 36±1°C for RS Coliform plates.

3. RESULTS AND DISCUSSIONS

Diffusible compounds production

The dual culture assay highlighted the presence of a clear inhibition zone between the two inoculated microorganisms. This indicate the ability of antagonistic yeast and bacterial strain to produce inhibitory diffusible compounds towards *B. cinerea* (figure 4).



B. cinerea control plate | Antagonistic yeast strain vs. *B. cinerea* | Antagonistic bacterial strain *P. fluorescens* vs. *B. cinerea* | Antagonistic bacterial strain *Bacillus subtilis* vs. *B. cinerea* | Microbial strain without antagonistic activity vs. *B.cinerea*

Figure 4. Antagonistic activity of the diffusible compounds released by the studied yeast and bacterial strains against B. cinerea. A – Control plate of B. cinerea, B-D – Dual culture test plates.

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

Inhibition of the *B. cinerea* mycelial growth was observed in all antagonistic tested strains studied by dual culture method. This is considered due to the microbial production of antifungal diffusible compounds released by the studied yeasts and bacterial strains. The inhibition rate varied between 42.44% and 69.63%. Best inhibition activity was recorded using *P. fluorescens* R2.1d, *P. chlororaphis* RG and *S. cerevisiae* L30 strains, which revealed an inhibitory efficacy of 69.63%, 64.70% and 65.36% respectively (table 2).

Table 2. In vitro testing of antagonistic strains efficacy (E%) against to Botrytis cinerea, through the production of inhibitory diffusible compounds

Antagonistic strain	E% of antagonistic diffusible compounds
Metschnikowia pulcherrima Sa5	56,91 ± 1,3 ^{abc}
***Saccharomyces cerevisiae* L30b**	**65,36 ± 0,9** ^{a}
Bacillus subtilis A4	59,76 ± 1,0 ^{ab}
***Pseudomonas chlororaphis* RG**	**64,70 ± 1,3** ^{ab}
P. fluorescens 79.3	47,78 ± 1,6 ^{bc}
***P. fluorescens* Rap2.1d**	**69,63 ± 0,3** ^{a}
P. fluorescens Rcp2	42,44 ± 0,8 ^{c}
Serratia plymuthica Ps33	49,98 ± 0,6 ^{bc}
Note: Results are presented as mean values ± standard deviation. Different letters indicate significant differences between experimental variants, at p = 0.05.	

VOCs production

The effect of microbial volatile compounds on *B. cinerea* mycelium growth was studied through the inverted plate method. The antifungal activity of VOCs is illustrated in figure 5.

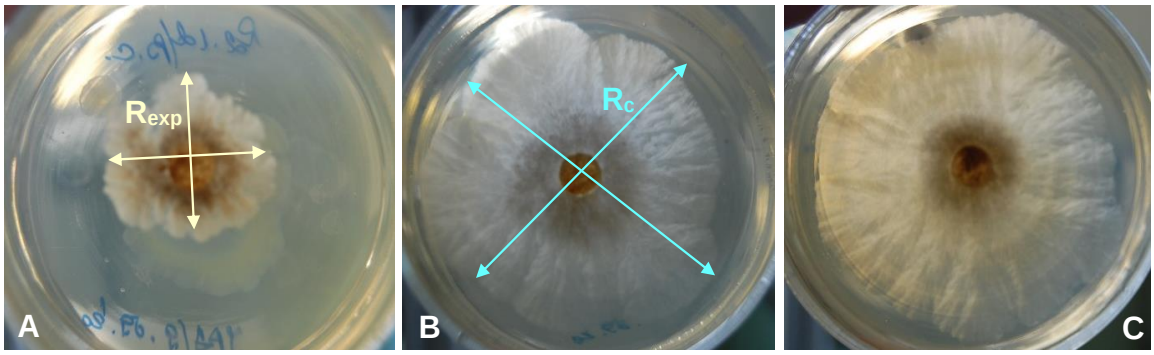


Figure 5. The effect of volatiles on pathogenic mycelium growth.
A – B. cinerea vs. Pseudomonas fluorescens R2.1d strain; B – B. cinerea control;
C – B. cinerea vs. non-volatile strain of Pseudomonas Rcp2.

Analyzing the antifungal activity of VOCs produced by the studied beneficial strains a strong inhibition activity was seen towards *B. cinerea* (table 3).

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

Table 3. In vitro antagonistic efficacy (E%) of microbial VOCs towards B. cinerea

Antagonistic strain	E% of VOCs
Metschnikowia pulcherrima Sa5	19.55 ± 5.8 ^{de}
Saccharomyces cerevisiae L30b	15.87 ± 16.6 ^{ef}
Bacillus subtilis A4	40.27 ± 6.0 ^{cd}
***Pseudomonas chlororaphis* RG**	**66.57 ± 2.9** ^{bc}
P. fluorescens 79.3	47.81 ± 2.8 ^{c}
***P. fluorescens* Rap2.1d**	**89.21 ± 1.7** ^{a}
P. fluorescens Rcp2	6.54 ± 3.2 ^{f}
***Serratia plymuthica* Ps33**	**74.87 ± 4.3** ^{b}

Note: Results are presented as mean values ± standard deviation. Different letters indicate significant differences between experimental variants, at p = 0.05.

Among the studied beneficial strains *Pseudomonas fluorescens* R2.1d, *Serratia plymuthica* Ps33 and *Pseudomonas chlororaphis* RG were highlighted with higher VOCs antagonistic activity. Their inhibition efficacy towards *B. cinerea* was 89.21%, 74.87% and 66.57%, respectively.

Evaluation of total and coliform microbiota on strawberry fruits

Microorganisms diversity is very high in nature, and can vary from one environment to another. Complex microbial consortia can be found naturally in soil, on plants, in water, and associated to humans and animals. When transferred by dust and aerosols, they are constant parts of the ambient air. During the food obtaining process, contamination of surfaces in the production environment is unavoidable. Good HACCP practices, well-conceived hygiene management as well as compliance with the general hygiene of the work space, are crucial factors to reduce the contamination risk of food products surface. Therefore, the correct performance of cleaning and sanitizing measures is absolutely important, but the periodic verification of successful disinfection is also important. The microbiological agars of the "RIDA-STAMP" product line are perfectly suited for the direct imprinting of surfaces in the production environment, and can be used as control tests to highlight the microbial load on food surfaces, including solid foods.

RS Total evaluation. Most of the microorganisms grown on the agar surface formed milky-white colonies, after 24 hours of incubation at 35°C (figure 6A). However, all visible colonies developed on the agar surface were counted and used for the quantification of total culturable aerobic, mesophilic microorganisms.

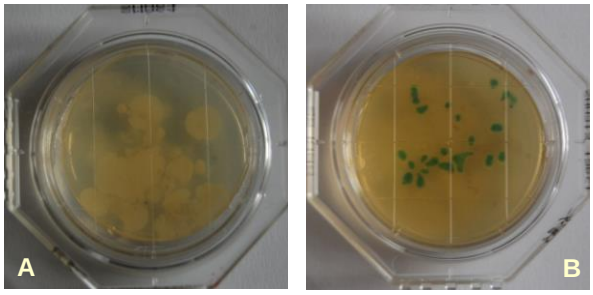


Figure 6. In vitro test for underline surface bacteria on RIDA-STAMP agar. A – Total culturable microorganisms; B – Coliform bacteria

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

Among the experimental variants, the highest microbial load on fruits surface was determined on strawberry collected from V3 experimental variant, were plants were grown on non-mulched matted rows (figure 7). Total microbial load in V3 was significantly higher compared to all other variants.
Evaluation of RS Coliform. All blue/green colonies grown on the agar surface were counted, to determine the total number of coliform bacteria (figure 6B). The highest microbial load of coliform bacilli was detected on fruits collected from V1 experimental variant, were plants were grown on non-mulched flat ground.
The analysis carried out under laboratory conditions on the contamination of strawberry fruits from organic culture, using RIDA STAMP agars (means on statistically calculated samples), showed a variable distribution regarding the total bacterial load (figure 7).

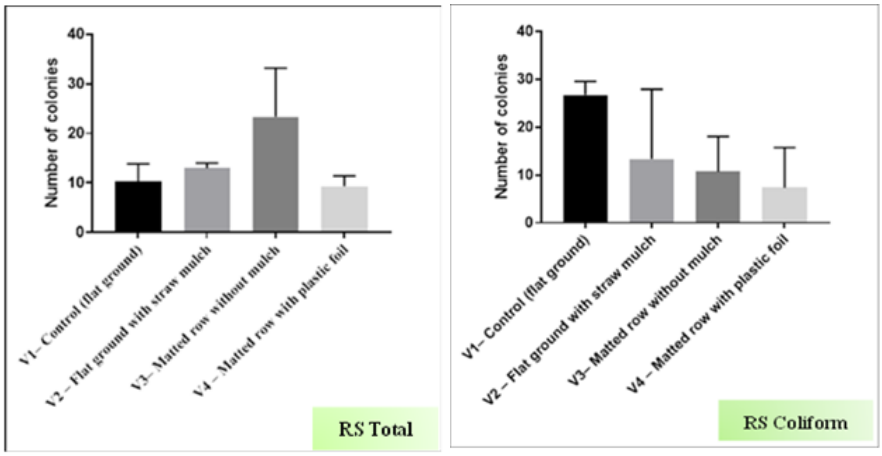


Figure 7. Diagram of strawberry fruits contamination with surface bacteria

A normal distribution, compared to the control variant, was observed in V4 experimental variant (matted row with plastic foil mulch) that presented the lowest microbial load. This aspect was due to the combination of matted row + plastic foil mulch, which act as a repressive factor for the development of some contaminating bacterial species. This was determined by the degree of hypoxia generated by the methodological combination, soil matting and plastic foil mulching. In terms of the coliform bacilli contamination, the V4 experimental variant was also statistically significant, having the lowest microbial load both compared to the control, with a significantly distinct difference compared to the other experimental variants.

4. CONCLUSIONS

Pseudomonas fluorescens R2.1d strain was able to inhibit *Botrytis cinerea* mycelial growth by releasing antifungal diffusible and volatile organic compounds. A 68.90% antifungal efficacy was shown by the antifungal diffusible compounds, and 88.9% efficacy was revealed by the VOCs.
The microbial load on organically produced strawberries was also evaluated. The lowest microbial load of coliform bacilli was shown on the strawberry fruits collected from the matted row variant, covered with black foil mulch. This way of strawberry cultivation assured a reduced microbial contamination of the fruits compared to the other technological option from the ecologic production system.

*Corresponding author, E-mail address: sicuia_oana@yahoo.com

Current Trends in Natural Sciences
Vol. 13, Issue 25, pp. 143-151, 2024
https://doi.org/10.47068/ctns.2024.v13i25.017
Current Trends in Natural Sciences (on-line) Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-953X ISSN: 2284-9521
ISSN-L: 2284-9521 ISSN-L: 2284-9521

5. ACKNOWLEDGEMENTS

This work was financed by the Ministry of Agriculture and Rural Development - MARD within the Project HG 4689 "Ecological solutions to reduce health risks in strawberry culture".

6. REFERENCES

Agrios, G.N. (2005). Plant Pathology. 5th ed. Department of Plant Pathology. University of Florida.

Arrebola, E., Sivakumar, D., Korsten, L. (2010). Effect of volatile compounds produced by *Bacillus* strains on postharvest decay in citrus. *Biological Control, 53* (1), 122-128, DOI: 10.1016/j.biocontrol.2009.11.010.

Boiu-Sicuia, O.A., Barbu, L.D.N., Diaconu, A., Paraschiv, A., Cornea, C.P. (2020). Beneficial yeasts with biocontrol potential against sweet potato storage pathogens. *Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. Ştiinţele Naturii., 36*(2), 34-45.

Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology*, *71*(9), 4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005

Huang, R., Li G., Zhang, J., Yang, L., Che, H. J., Jiang, D. H., Huang, H. C. (2011). Control of postharvest *Botrytis* fruit rot of strawberry by volatile organic compounds of *Candida intermedia*. *Phytopathology, 101*(7), 859–869.

Lopes, M. R., Klein, M. N., Ferraz, L. P., da Silva, A. C., & Kupper, K. C. (2015). *Saccharomyces cerevisiae*: A novel and efficient biological control agent for *Colletotrichum acutatum* during pre-harvest. *Microbiological Research, 175*, 93–99. https://doi.org/10.1016/j.micres.2015.04.00

Maas, J. L. (1998). Compendium of strawberry diseases. St. Paul (MN): APS press.

Magnin-Robert, M., Quantinet, D., Couderchet, M., Aziz, A., Trotel-Aziz, P. (2013). Differential induction of grapevine resistance and defense reactions against *Botrytis cinerea* by bacterial mixtures in vineyards. *BioControl, 58*, 117–131. https://doi.org/10.1007/s10526-012-9474-y

Mangunwardoyo, W., Aprilismulan, A., Oetari, A., Sjamsuridzal, W. (2011). Screening cellulose activity of yeast isolated from soil, sediment and water river from Taman Nasional Gunung Halimun, West Java, Indonesia. *Malaysian Journal of Microbiology, 7* (4), 210-216. https://doi.org/10.21161/mjm.02511

Oancea, F., Ştefan, A.L., Lupu, C. (2015a). *Tulpina de Saccharomyces cerevisiae cu activitate antagonista si fermentativa*. [*Saccharomyces cerevisiae strain with antagonistic and fermentative activity*] (Patent No. RO127523) Oficiul de Stat pentru Inventii si Marci. https://osim.ro/wp-content/uploads/Publicatii-OSIM/BOPI-CBI-BI-EU/2015/ext_02_2015.pdf

Oancea, F., Dinu, S., Constantinescu, F., Sicuia O. (2015b). *Tulpina de Serratia plymuthica cu actiune antagonica fata de fitopatogenii plantelor de cultura*. [*Serratia plymuthica strain with antagonistic action against plant pathogens*.] (Patent No. RO127469) Oficiul de Stat pentru Inventii si Marci. https://osim.ro/wp-content/uploads/Publicatii-OSIM/BOPI-CBI-BI-EU/2015/ext_02_2015.pdf

Spadaro, D., Droby, S. (2016). Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Science & Technology, 47*, 39–49.

Zhang, H., Wang, L., Dong, Y., Jiang, S., Cao, J., Meng, R. (2007). Postharvest biological control of gray mold decay of strawberry with *Rhodotorula glutinis*. *Biological Control, 40* (2), 287–292. https://doi.org/10.1016/j.biocontrol.2006.10.008

*Corresponding author, E-mail address: sicuia_oana@yahoo.com