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ORIGINAL PAPERS

INNOVATIVE SOLUTIONS FOR PLANT PROTECTION BASED ON VEGETAL EXTRACTS AND LEATHER WASTE CAPITALIZATION – APPLICATION ON STRAWBERRY CULTURES

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Abstract. *Innovative solutions in the decisive control of pests and elective treatments to stimulate agricultural production will accomplish the need to feed a growing global population and the long-term toxicity of synthetic fungicides. The product development is based on indigenous potential of natural antifungal compounds (eg vegetable extracts from fenugreek and marigold, tomato glycoalkaloids, etc.) as well as on circular bio-economy - obtaining protein hydrolysates from tannery waste. In this economic niche context, an innovative bio-fungicide and bio-stimulator prototype was created, with enhanced effectiveness through the profile and ratio of the associated active compounds, with an extended spectrum of action, GLYCAM-STIM Combo. The impact of two variants of biopesticide was compared to the commercial ecological product Serenade, on strawberries - Premial variety. It was investigated the development of gray rot - Botrytis cinerea and the effect on the production and quality of the* *fruits. The results demonstrate the limitation of the frequency of the attack and its intensity, the increase of fruit firmness and mass (by 8g) and the production's rise by 7t/ha. The data confirm the applicability's extension of the multifunctional biopesticide also at the level of the strawberry species, as well as in the protection of cherry orchards. The research was carried out within the project BIO-PLANT-PROTECT 262 / 2021.*

Keywords: biopesticide, plant biostimulator, marigold, fenugreek, Botrytis cinerea, strawberries

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1. Introduction

The pesticide concept had a significant evolution over time, starting from the product's efficacy, but with a significant focus on the ecological impact. A large number of pesticides have been withdrawn from the market due to high toxicity, long decomposition time, improper application methods. Many cases of chronic or acute toxicity and increased resistance of target species, replacement of target species with resistant pest species and environmental contamination have been reported. (1) Considering all these aspects, a series of properties of the products under development are required to ensure a proper toxicity / efficacy balance: high selectivity on target species, low toxicity on non-target organisms; high effectiveness using reduced amounts of pesticides; low persistence in the environment (rapid decomposition) to avoid bioconcentration and biomagnification in the food chain, in order to prevent the development of resistance in organisms.

The use of biopesticides began at the end of the 1800s, when fungal spores were used to control insect infestations. (2). Botanical pesticides have gained great importance in recent years, through the development of research on plant sources, estimating over 6500 species of plants with active potential, with different combinations of chemical compounds, from over 240 families. (3) Different species can be mentioned, e.g. Chrysanthemum cinerariaefolium, Chrysanthemum coccineum, Haloxylon salicornicum, Stemona japonicum, Schoenocaulon officinale, Origanum vulgare, Thymus vulgaris, Azadiractina indica, Leuzea carthamoides, Mentha spp., Lavendula spp., Nicotiana spp., containing polyphenols, α - chaconine, nicotine, thymol, carvacrol, pyrethrins, rotenones, with direct action, through multiple mechanisms, on various species of insects, fungi or bacteria that destroy the crops. [4]. (5)

The fenugreek extract (Trigonela foenumgraecum), having a phytochemical content of phenolic compounds, flavanols and flavones, alkaloids and pro-anthocyanidins, has a strong inhibitory effect on micellar growth. The antimicrobial action is dependent on the content of these active principles, growing conditions and the harvest period, the vegetative stage being the richest in active principles. Experimental results demonstrate the antifungal activity of the aqueous extract on pathogenic fungi of tomato plants. (6), (7)

From the perspective of the configuration of a biofertilizer, it is important to insist on the sustainable valorization of resources and the conversion of biomass into bio-products. Reducing the use of synthetic pesticides is possible only by maintaining the productivity and quality of crops. Organic farming is practically non-toxic and leads to much less residue, but it can be about 25% less productive. (8)

The use of green agro-products impacts the natural conservation principles, environmental sustainability, and judicious use of critical resources. These goals are reflected in green chemistry, which aims to mitigate harmful chemicals from production down to application, process safety, optimal energy consumption, and improved economy (9)

Our experimental design will focus on a complete solution for the support and protection of agricultural crops: anti-pest action (insects, fungi, etc.) and fertilizers for seeds, applying circular economy approaches. We target the association of plant extracts with biopesticide properties. The following species are considered: Trigonela foenumgraecum, Tagetes patula, with extracts from fam. Solanaceae and Asteraceae/Fabaceae, camelina oil and biostimulants resulting from keratin and collagen hydrolysates from the leather industry. Regarding protein waste from the leather industry, we have in mind previous application on processing and biorefining on several levels, obtaining fractionated colagen, keratine and gelatine. The study is the final part of the biopesticide's efficacy validation in a real system, an experimental strawberry culture. It was performed after previous confirmation on „in vitro" studies on targeted pathogen strains and multiple action on seeds germination and antioxidant protection. (10), (11)

The strawberry crops are well appreciated and commercially valuable, but is continually subject to various risks, one of the most serious of which is the diseases caused by phytopathogenic organisms. More than 50 different genera of fungi can affect this cultivar, including Botrytis spp., Colletotrichum spp., Verticillium spp, and Phytophthora spp. (12) These pathogens, single or in combination, fill the vascular system of strawberries, resulting in the prevention of nutrient and water uptake and causing root blackening, root death, and a loss of the vitality and productivity of the plant (13)

We proposed an original biopesticide formula as a control strategy of Botrytis spp. atack and biostimulator of fruits quality parameters addressed to limit the economic losses of the strawberry crop.

2. Materials and Methods:

2.1. Active compounds for testing - Associative formulas of biopesticides:

- GLYCAM - STIM combo I - GLY T (tomato glycoalkaloids) + camelina oil + Ska fenugreek butylene glycol extract + CHC3B protein hydrolysate (biopesticide with ambivalent, antifungal and fertilizing activity);
- GLYCAM - STIM combo II - GLY T (tomato glycoalkaloids) + camelina oil + marigold butylene glycol extract + protein hydrolysate CHC2B (biopesticide with ambivalent, antifungal and fertilising activity);
- GLY Cam – Plus – GLY T (tomato glycoalkaloids) + camelina oil + Ska fenugreek butylene glycol extract

Standard product – experimental control - Serenade® ASO - 3l/ha

Experimental lots encode:
V1 = Untreated control
V2 = **GLY Cam Stim Combo I (**GLY T I) – 1,0%
V3 = **GLY Cam Stim Combo II** (GLY T II) – 1,0%
V4 = **GLY Cam Plus** (GLY T) – 1,0%
V5 = standard product (Serenade® ASO) - 3l/ha

2.2. Experimental Assesment Conditions

Experimental field – Research&Development Institute for Fruit Culture - Pitesti - Mărăcineni (ICDP Pitești-Mărăcineni).

Culture (species, varieties): strawberry, variety: Premial.

Experimental doses: 1 dose (one concentration): 1.0%.

Target pathogen: gray rot - Botrytis cinerea.

Aim of the study: Effect of biopesticides application on fruit production and quality

Experimental design for field observations: 3 repetitions in a completely randomized block, 3 repetitions in a randomized block, 25 plants/repetition – 75 plants/variant; Repeat length: 7.5 m; Repeat width: 0.8 m; Surface variant: 18.0 m2

Plot size, number of plants: 90.0 m2, 755 plants
Planting distance: 0,3 x 0,8 m
Method and time of product application: foliar spraying.

Stages of treatments:
I: Elevation of inflorescences
II: the first open flowers
III: fruits formed
IV- the beginning of the specific coloring of the first fruits

V – after the first harvest

The analyzes were performed in accordance with the Norms EPPO:PP 1/16(2) Botrytis cinerea on strawberry and EPPO:PP 1/135(4) Phytotoxicity assessment

Results and discussion

Analyzing the meteorological data recorded in the Mărăcineni - Argeş area in the last 55 years, we notice that the average annual temperature during this period (10.1˚C) was one that was within the range specific to the species studied (9 - 11˚C), favorable climatic conditions. At the level of the entire studied period, the pluviometric deficit reached a value almost three times higher than normal (311 mm, compared to normal by 93 mm), which could lead to serious nutritional deficiencies with consequences on the fruiting process the following year. It has to be mention that climatic conditions dramatically impact the rise and the quality of fruits, as well as the pathogen attack. The risk of attack by the pathogen Botrytis cinerea can be observed from the end of February and from the first decade of May, being associated with the long duration of the number of hours of wetting of the foliage. (fig.1, 2)

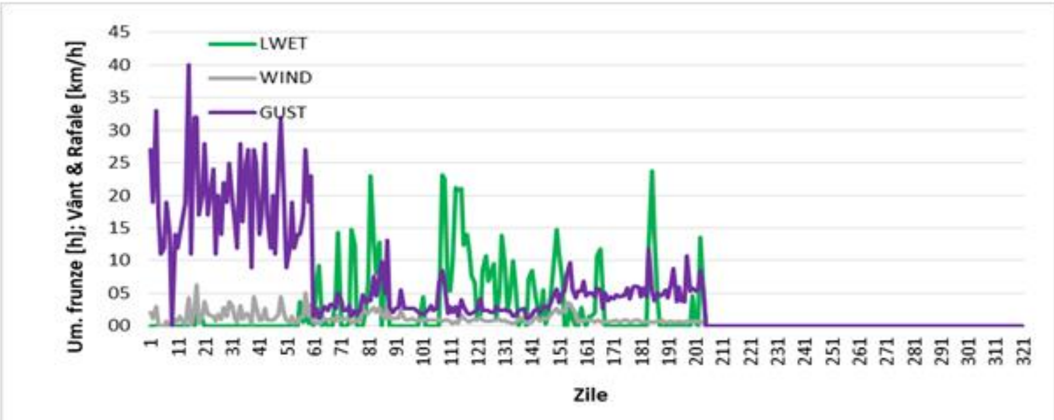

Fig. 1: Duration of wetting of foliage, wind speed and gusts, ICDP Pitesti-Mărăcineni, 2024

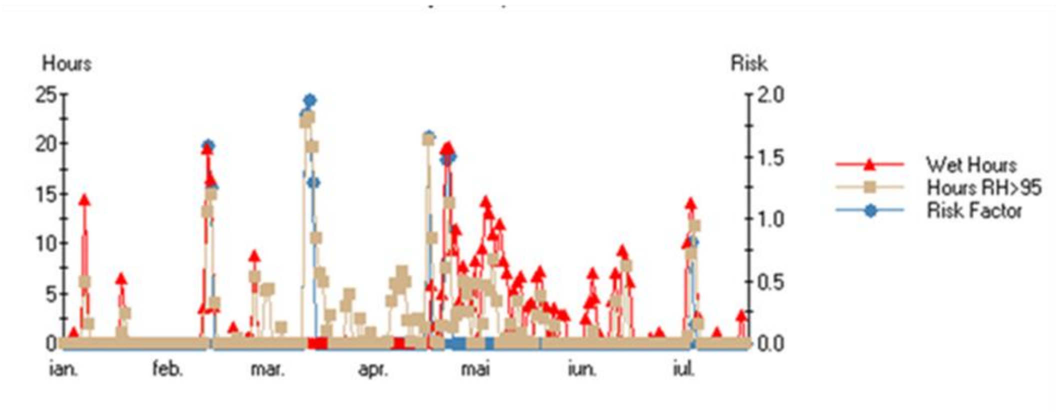

Fig. 2: The risk of infections with gray rot - Botrytis cinerea, ICDP Pitesti Maracineni, 2024

A. Biological efficacy of biopesticides GLY Cam Stim Combo I, GLY Cam Stim Combo II and GLY Cam Plus in the control of strawberry gray rot (Botrytis cinerea)

To determine the attack caused by the gray rot of the strawberry - Botrytis cinerea, observations were made on 25 marked plants in each repetition, noting the frequency (F%) and the intensity of the attack (I). Based on the collected data, the following were calculated:

The degree of attack according to the formula GA% = [F% x I (1-3)]/1000

Product efficacy according to the Abbott formula where:

E% = [1-(A/B) x100], where A represents the GA% of the treated variant and B the GA% of the untreated control variant.

The biological efficacy of the tested products was presented in the following figures and tables, in a synthetized manner from field observations.

BIOPESTICIDES	Gray root frequency on fruits F%	Attack degree (%)	Efficacy (%)
V1-Untreated control	30	0.91	0
V2-ComboI	13.38	0.31	55.65
V3-ComboII	15.33	0.36	49.19
V4- GlyCam Plus	10.67	0.25	64.63
V5-Standard Serenade	8.42	0.17	72.09

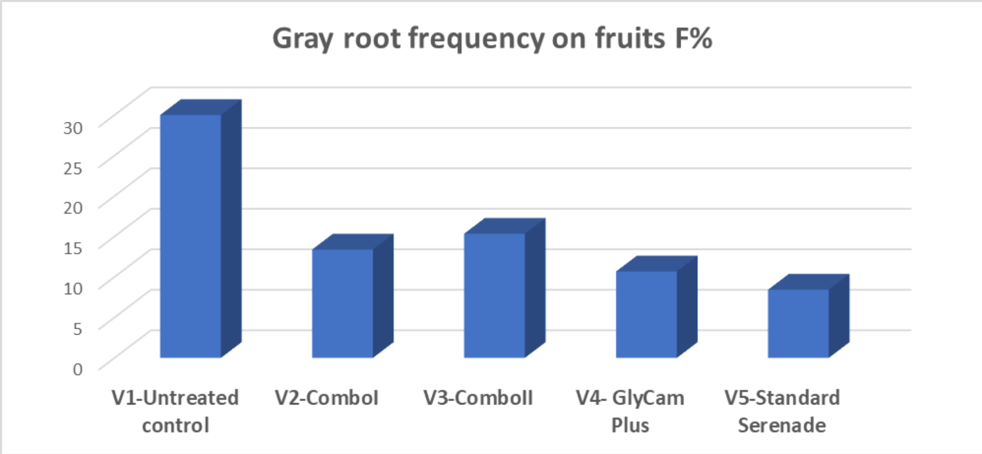

Fig.3: Frequency of gray rot (Botrytis cinerea) attack on strawberry

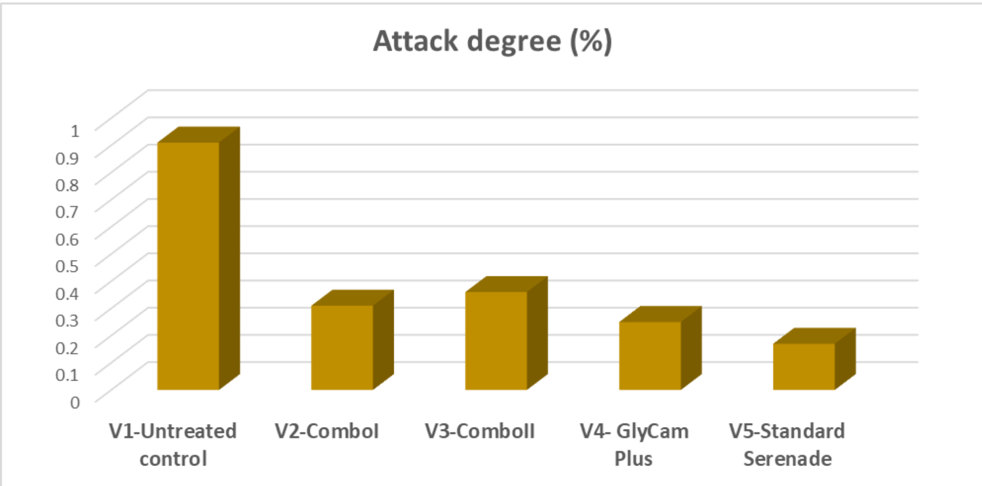

Fig.4: Decrease of gray rot (Botrytis cinerea) attack degree on strawberry's culture.

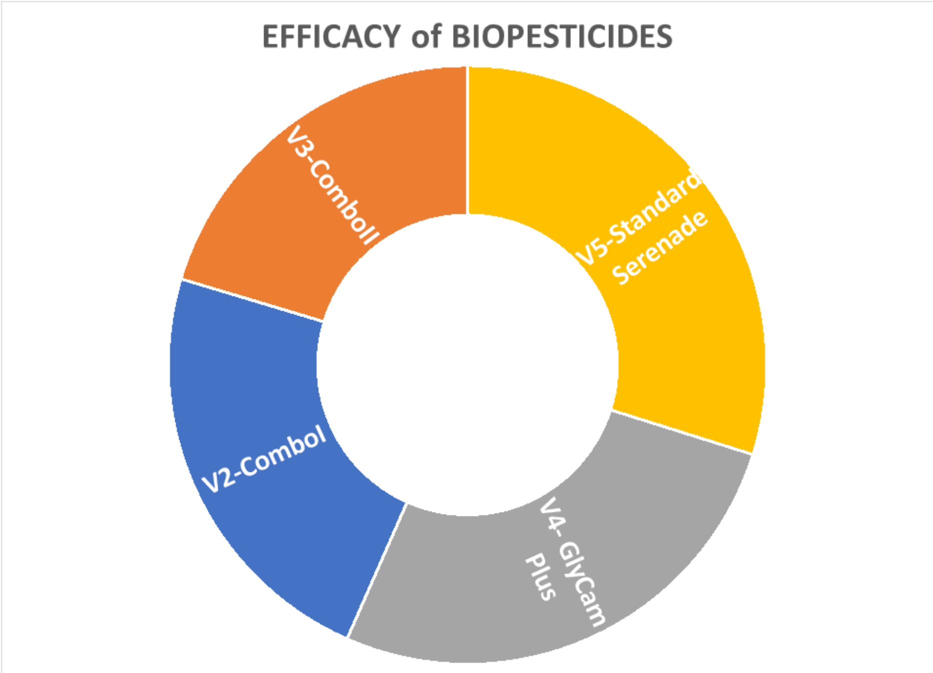

Fig. 5: The decrease of pathogenic attack and the efficacy in plant protection induced by biopesticides

Among the biopesticides tested, the highest efficacy (64.63%) combating strawberry gray rot (Botrytis cinerea) was obtained in the case of variant V4, treated with the GLY Cam Plus biopesticide, applied at a concentration of 1%. Also, an efficacy of over 50% was obtained in the case of the V2 variant, treated with GLY Cam Stim Combo I (55.65%), applied at a concentration of 1%.

B. The effect of applying the biopesticides GLY Cam Stim Combo I, GLY Cam Stim Combo II and GLY Cam Plus on fruit production and quality

The observations were of numerical type, with the aim of obtaining data and characteristic parameters for the objective evaluation. The statistical method used to test the differences between the averages of the determined indicators was the analysis of variance, and the differences between the variants were highlighted using the Duncan test for a confidence level of α=0.05. The data were processed using the SPSS 14.0.0 program.

As shown in the table and figures below, in all variants in which treatments were applied, the weight of the fruits was significantly higher compared to the untreated control variant, (19.8-20.3 g, compared to 12, 8g).

BIOPESTICIDES	Weight of fruits(g)	Firmness of fruits (HPE IIFFF Bareis units)	Fruits production (t/ha)
V1-Untreated control	12.8	31.2	10.99
V2-Combol	19.8	40.2	17.75
V3-ComboII	20.3	39.4	17.14
V4- GlyCam Plus	19.2	38	15.29
V5-Standard Serenade	18.6	36.8	16.64

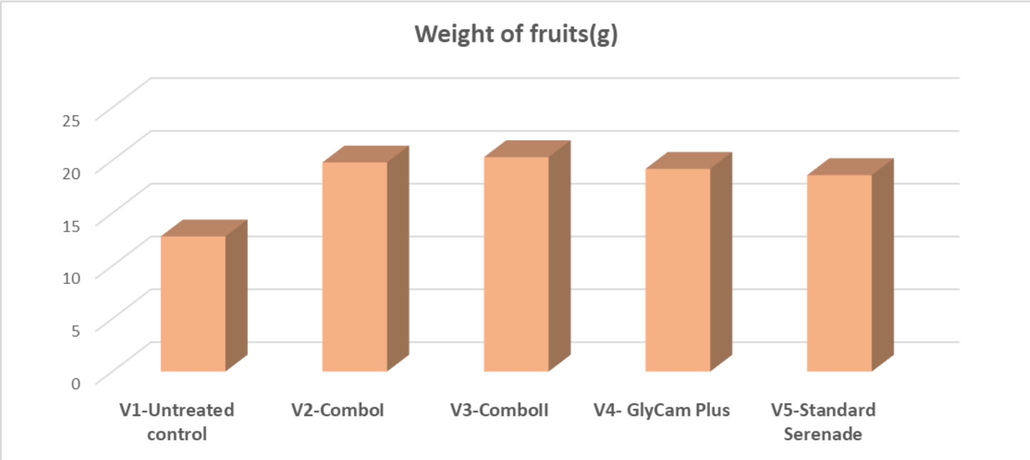

Fig. 6: The influence of fertilization options on fruit mass - strawberry species, Premial variety

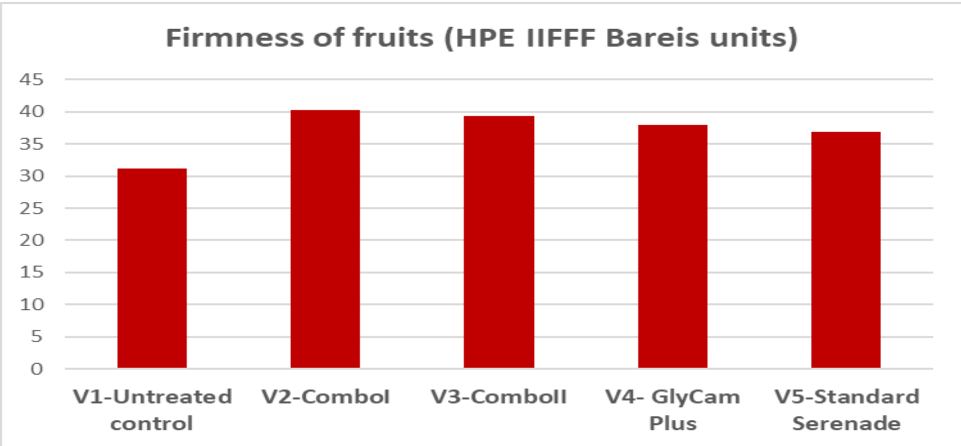

Fig. 7: The influence of fertilization options on fruit firmness - strawberry species, Premial variety

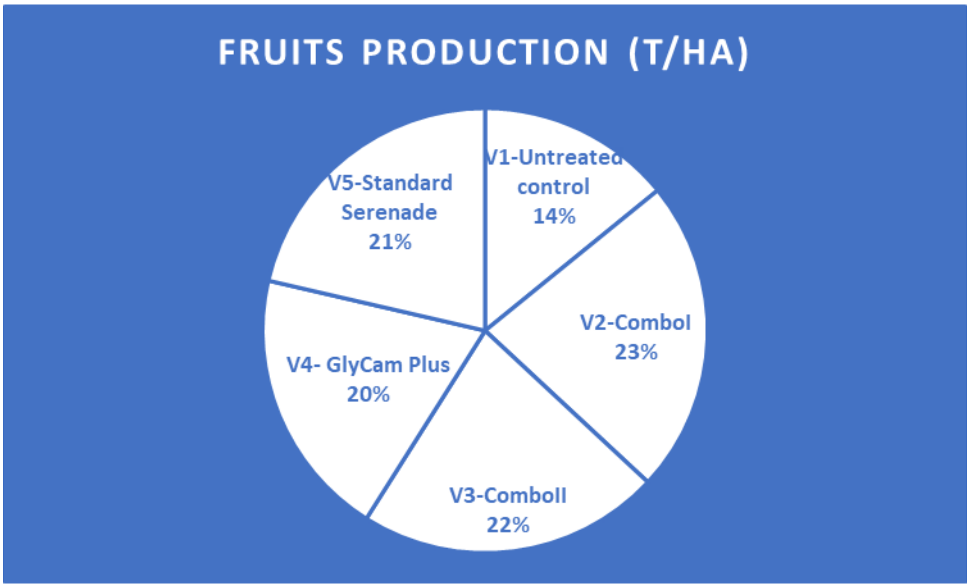

Fig. 8: The influence of fertilization options on fruit production - strawberry species, Premial variety

The analysis of the differences between the fruit firmness of the five compared variants indicated that, except for the V4 and V5 variants (treated with GLY Cam Plus and respectively the Serenade ASO standard), with 38.0 and 36.8 HPE II FFF Bareiss units respectively, the harvested fruits from variants V2 and V3 stood out for their significantly higher firmness compared to the control variant (V1). The V2 variant, treated with GLY Cam Stim Combo I, stood out in particular, with a fruit firmness of 40.2 HPE II FFF Bareiss units.

CONCLUSIONS

The phytochemical potential of different plant species (fenugreek, marigold, camelina, tomatoes) was exploited for antifungal and fertilization efficacy, as an important step for a plant protection product development. Our studies highlighted the multivalent activity of several combination of active principles acting as antifungal and harvest stimulants. The limitation of the frequency of the gray rot attack and its intensity, the increase of fruit firmness and mass (by 8g compared to the control) and the production of strawberries by 7t/ha compared to the untreated control were demonstrated. The data confirm the extension of the applicability of the multifunctional biopesticide also at the level of the strawberry species, after the reported success in the protection of cherry orchards and a good "in vitro" - "in vivo" correlation of the antifungal tests.

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