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Case Study: Innovative Solutions for the Management and the Sustainable Phytosanitary Control on *White Onion of Margherita IGP*

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Abstract

All the farms follow the monocropping system, allocating the entire area to a single species, i.e. Allium cepa L., in particular to the local ecotypes Marzaiola and Aprilatica (early maturity types), Maggiaiola (medium-early maturity type), Giugliese and Lugliatica (late maturity types).

It must be borne in mind that such an intensive crop, in specialized areas, and with a restricted number of species as it is our case, must primarily focus on the crop protection.

Furthermore, consumers are increasingly attentive to the consumption of healthy products, with high nutritional value and environmental sustainability. This requires new development models for the entire food supply chain that follow principles of environmental and social sustainability to preserve non-renewable natural resources (soil, water, air, biodiversity, and energy sources).

The experience gained thanks to the project “Innovations for the improvement of the production potential of the White Onion of Margherita IGP” (CIPOMAR), funded by the Apulia Region, PSR Puglia call for bid 2014-2020- sector 16 – Cooperation- Sub sector 16.2 “support to pilot projects and to the development of new products, practices, processes and technologies” has enhanced the potential and the challenges relevant to the production sustainability.

The activities concerned the identification of strategies for the management and control of adversities, which reflect the concept of environmental sustainability, aimed at guaranteeing a quality product, trying to further enhance the organoleptic and nutritional characteristics typical of the product, as well as quantitative.

Keywords: Solilborne pest; Management; Sustainable; Fusarium; Nematodes; Onion

Introduction

It has been taken into consideration the overall objective to promote and improve the profitability, the competitiveness, and the sustainability of the production and supply chain of the White Onion of Margherita IGP.

The farms who specialize in the traditional cultivation of the White Onion of Margherita IGP are located in the municipality of Margherita di Savoia – province of Barletta-Andria-Trani, the municipalities of Zapponeta and Manfredonia – province of Foggia (all in the Apulia Region, Italy).

All the farms follow the monocropping system, allocating the entire area to a single species, i.e. Allium cepa L., in particular to the local ecotypes Marzaiola and Aprilatica (early maturity types), Maggiaiola (medium-early maturity type), Giugliese and Lugliatica (late maturity types).

The uniqueness of this produce is strictly linked to the agro-ecosystem where it is grown, characterized by the influx of the sea streams, the presence of salt ponds, the winds blowing from the Gargano mountains, and the existence of a sandy soil that on one side allows a regular and uniform development of the bulb, and on the other enhance the early maturity of the crop by easily heating in the springtime.

It must be born in mind that such an intensive crop, in specialized areas, and with a restricted number of species as it is our case, must primarily focus on the crop protection.

The experience gained thanks to the project "Innovations for the improvement of the production potential of the White Onion of Margherita IGP" (CIPOMAR), funded by the Apulia Region, PSR Puglia call for bid 2014-2020- sector 16 – Cooperation- Sub sector 16.2 "support to pilot projects and to the development of new products, practices, processes and technologies" has enhanced the potential and the challenges relevant to the production sustainability.

The repeated cultivation of onion in the same soil for several years leads to an increase in harmful biological factors in the soil (Soil-borne pest): a selection of animal and plant parasites specific to the cultivated species, pathogens of the root system and weeds that are difficult to control, as well to a decrease in soil fertility.

Furthermore, consumers are increasingly attentive to the consumption of healthy products, with high nutritional value and environmental sustainability. This requires new development models for the entire food supply chain that follow principles of environmental and social sustainability to preserve non-renewable natural resources (soil, water, air, biodiversity, and energy sources).

Sustainable agriculture, especially organic agriculture, is based on four fundamental principles: health, ecology, equity and precaution [1]. The objective is to combine innovation and research with the well-being of people and the environment, following a holistic approach.

Therefore, the project aims to promote and improve the profitability, the competitiveness and the sustainability along the entire production chain of the Cipolla Bianca di Margherita IGP, in order to produce healthier and safer foods and to increase the resilience of individuals and ecosystems. This brings plant protection to the foreground to limit environmental pollution.

The activities concerned the identification of strategies for the management and control of adversities, which reflect the concept of environmental sustainability, aimed at guaranteeing a quality product, trying to further enhance the organoleptic and nutritional characteristics typical of the product, as well as quantitative.

Furthermore, to the difficulty of controlling some agents of damage is added the worrying public opinion towards the problems linked to the use of chemical products, in addition to the existing regulatory constraints. Therefore, it was deemed appropriate:

- To evaluate the susceptibility of White Margherita Onion selections to biotic and abiotic adversities (Task 3.3).
- To determine an adversity management program with full respect for the operator, the environment, and the consumer (Task 3.8).

The control of phytosanitary problems represents an issue that must be addressed with the greatest attention and accuracy, as the production area of the "White Onion of Margherita" is located along the Adriatic coastal strip, delimited by the sea and the largest salt ponds in Europe. The "Margherita di Savoia Salina Wetland" is a largely uncontaminated protected area, declared an area of international importance under the Ramsar Convention, According to Legislative Decree 150 of 2012, which represents the transposition of EUROPEAN DIRECTIVE 128 of 2009, in particular Art. 15 (Reduction in the use of plant protection products or risks in specific areas) it is appropriate to develop a strategy for controlling the crop adversities while respecting the concept of environmental sustainability. Furthermore, the growing sensitivity of markets and consumers towards low residue products puts the farmers and technicians operating in the sector in the position of having to look at alternative solutions.

Materials and Methods

Monitoring and Phytosanitary Assessment for Correct Management of Adversities

The study and monitoring of the activities in some farms in the Margherita di Savoia countryside (southern Italy) began in July 2020, which made it possible to take note of the characteristics of the crops, detecting the phytosanitary state and collecting several samples. Particular attention was paid to identifying any symptomatic differences (Figure 1). Therefore, after a preliminary study phase to acquire more information on potential patho-systems, in order to determine a general picture of the various plant diseases that afflict the onion crops, in that particular context, not only pedoclimatic but also agronomical (different ecotypes), we subsequently developed guidelines for the management of the respective adversities detected.

Figure 1: Monitoring the phytosanitary status of the crops

The surveys during the various phenological phases of the crops were also carried out in 2021, by checking the appearance of primary infections of the various pathogens and the outcome of the infections. The plant material that revealed itself to be symptomatic at first sight was collected and, from time to time, moved to the laboratory, to carry out the analyses that eventually would lead to the identification of the pathogen possibly present on it. The collected samples, once brought to the laboratory, were subjected to macroscopic and microscopic observations, as well as to a mycological analysis of the altered parts (leaves, bulbs, and roots), through the isolation of the fungal species on Petri dishes.

The method involved an initial washing phase of the tissues to be analyzed with running water, in order to remove the adhered soil and after drying them, they were disinfected using the technique described in the protocol of Fisher et al. (1992), which involved an immersion in 75% ethyl alcohol for 60 seconds, followed by an immersion in a 4% active chlorine sodium hypochlorite solution for 60 seconds, and finally a final immersion in 75% ethanol % for another 60 seconds. Once this process was completed, the material was carefully dried with sterile bibulous paper and subsequently sectioned into smaller fragments (approximately 0.2-0.3 cm3). These fragments were placed on Agar-Potato-Sucrose (APS) based substrates with the addition of 200ppm streptomycin sulphate and 100ppm neomycin sulphate. The inoculated Petri dishes were incubated at 25°C in the dark for 15 days. From the fungal colonies developed on the isolation substrate, pure colonies were obtained to subject them to morphological characterization and identification studies (Figure 2). Observations under the optical microscope (Axiophot – ZEISS) and micrometric measurements were carried out on "wet" preparations mounted in a mixture composed of 2 parts water and 1-part lactic acid.

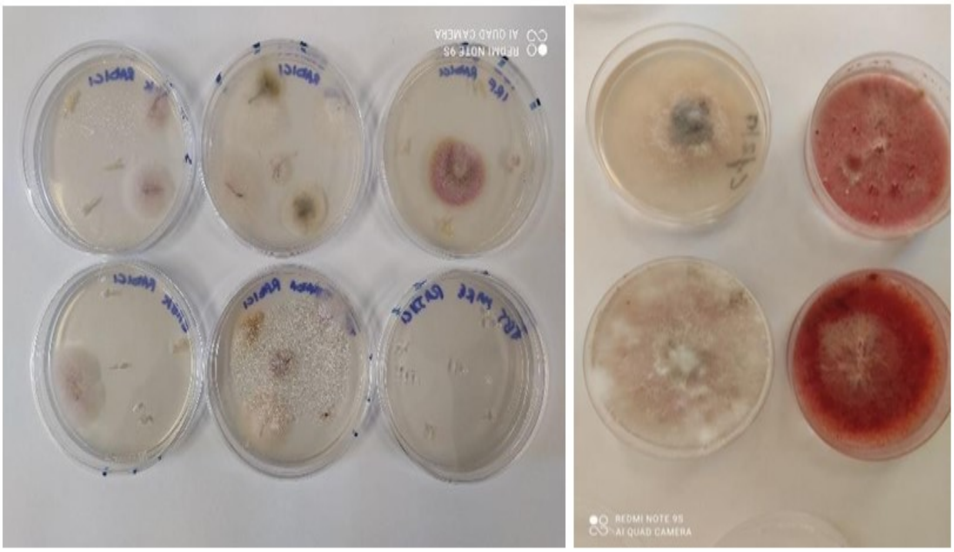

Figure 2: Isolation and identification of fungal species from onion tissues

To verify the presence of pathogens with edaphic habitus, isolations were also carried out from soil samples. The soil samples to be analyzed, consisting of elementary samples taken at random (eliminating the first 2-4 cm of soil and taking the underlying soil to a depth of 20-30 cm), were carefully and uniformly mixed, and placed inside polyethylene bags labeled with the collection date and farm, to be brought to the laboratory. In the laboratory, the number of microorganisms, or more precisely the number of Colony Forming Units (CFU) per gram in each soil sample was determined by plating successive dilutions starting from an initial suspension. Subsequently, pure colonies were obtained for further morphometric characterization studies.

Additionally, 100g soil samples were taken from each individual area and examined to extract nematodes from the soil using the Baermann funnel technique. The technique uses a glass funnel with a diameter of 10-15 cm, to which a rubber tube closed by forceps is connected. The funnel is positioned vertically on a support and filled with water until it touches a bag or a net containing the soil under examination. The nematodes, due to their marked hygrotropism, migrate from the soil to the water and gather at the bottom of the pipe [2]. To identify the presence of nematodes in plant samples, a procedure was used to extract nematodes from the plant tissues themselves. The procedure involved placing the plant fragments in a small quantity of water, placing the container under the binocular, tearing the plant tissues with dissecting needles, and directly observing the presence of nematodes.Furthermore, the observations were not limited to open field cultivations, but also involved fields intended for the production of seed-bearing bulbs and seed-bearing crops, including those created under cover (nonwoven tunnels), set up specifically in Castelluccio dei Sauri (41.30613667446456, 15.500558954706422), in an isolated area never involved in onion cultivation. The objective was to evaluate the variation in response to different pathogens of individual onion varieties.

Identification of the Intervention Strategy

After careful evaluation of the phytosanitary conditions of the crops, the study phase aimed at identifying the intervention strategy to be adopted was started. In order to mitigate the negative effects of edaphic microorganisms, a field experiment was conducted at the Giannino Antonio farm located in the Orno area in the countryside of Margherita di Savoia (BT), (41°23'36'' N; 16°6'42''E), in spring 2021.

The objective of this activity was to compare different plant protection products, both biological and chemical, in order to develop an effective strategy for the control of pathogenic microorganisms. The experimentation involved four plots of 10 m2 each, with three replications according to the experimental scheme based on a randomized block of 4 theses (Table 1).

The transplanting of the seedlings, produced in nurseries directly in the field, took place on 20 April 2021.

After 60 days from the transplant, no significant differences emerged between the different treatments, but at 100 days the complete ineffectiveness of the treatments was noted, to the point that the seedlings of the control group, although symptomatic, showed less serious symptoms.

The causes of this ineffectiveness can be attributed to the use of already compromised propagation material.

In 2022, the effectiveness of the "Anaerobic Soil Disinfestation" (ASD) technique was evaluated. This methodology involves the combined use of organic amendments and solarization in conditions of water saturation of the soil. Solarization uses solar energy to raise the temperature of the soil, causing a significant reduction in pathogens present in the surface levels of the soil, generally occupied by the root system of plants.

Table 1: Open field trial run in 2021 – "Farm Giannino Antonio"

Thesis	Active ingredient	**Commercial product**	**Mode of application**
Thesis 1	- Metam sodium	METHAM NA 51 (TAMINCO ITALIA S.r.l.)	Mechanical application (soil injection by a pole) thirty days before transplant of the onion seedlings at a rate of 300 l/ha
Thesis 2	Metam sodiumBrassicacee powder*Trichoderma harzianum T-22*	-METHAM NA 51 (TAMINCO)-PelletsBiofence (TRIUMPH)-Trianum-P, (Koppert)	Mechanical application (soil injection by a pole) thirty days before transplant of the onion seedlings at a rate of 300 l/haSoil incorporation at 15 cm depth, followed by uniform irrigation) seven days before transplanting of the onion seedlings at a rate of 400-600 g/m^2soil application at transplanting time through fertigation at the rate of 5 Kg/ha
Thesis 3	Brassicacee powder*T. harzianum T-22*	PelletsBiofence (TRIUMPH)Trianum-P, (Koppert)	Soil incorporation at 15 cm depth, followed by uniform irrigation) seven days before transplanting of the onion seedlings at a rate of 400-600 g/m2soil application at transplanting time through fertigation at the rate of 5 Kg/ha
Thesis 4	Untreated		

Organic manure in soil subjected to solarization implements the process known as bio solarization, which enhances the effect of solar heat and shows more evident results against soil fungi and nematodes [3, 4, 5, 6, 7]. ASD proves to be effective thanks to several factors, including the lack of oxygen, the accumulation of toxic products deriving from anaerobic decomposition (organic acids, methyl sulfides, carbon dioxide, methane, nitrous oxide) and antagonism by anaerobic organisms [8].

The experiment conducted in the open field at the Azienda Nuova Agricoltura (41.398618, 16.090816) was implemented - previous covering the ground with polyethylene and abundant irrigation (Figure 3) - by the distribution and subsequent burial of the pomace both in the plots designated for coverage than in those with no cover, in order to implement two experimental theses: thesis 1 - solarization and organic soil improver; thesis 2 - only organic soil improver. The two experimental theses were compared with a third untreated thesis.

The same year, another organic management trial was conducted on the same farm, which involved the application to the soil of fumigants of natural origin (Allyl isothiocyanate - Senapoil - SKL), resistance inducers (Ep5-Protect) and rock dust, in addition to the use of mycorrized plants with arbuscular mycorrhizal fungi (FMA) obtained through nursery applications of mycorrhizal fungi and Trichoderma artroviride (Team mix) (Table 2).

Figure 3: Setting up ASD trials on the farm “Nuova Agricoltura”

Table 2: Open field trial run in 2022 – “Azienda Nuova Agricoltura” with seedling from nursery

Thesis	**Active ingredients**	**Commercial product**	**Mode of application**
Thesis 1	untreated		
Thesis 2	MychorizlfungiandTrichoderma atroviride	Team mix	Nursery applications by mixing the inoculum to the substrate
Thesis 3	Isotiocianato di allile (rapeseed oil)	*Senapoil* - SKL	Soil application with injection pole seven days before transplanting of onion seedlings at a rate of 325 l/ha
Thesis 4	Resistance inducers(Ep5-Protect” and rock powder)	ELVISEM Indústria	Sprayed on the soil before and after transplanting at a rate o 6 g/ha in 30 l of water.

In 2023, a new field trial was carried out at the Giannino Antonio farm (41°23'36"N; 16°6'42"E), following the intervention plan identified in the previous trials.

On 17 January 2023, the application of Isothiocyanate - Senapoil-SKL (5 liters per 1000m2) was carried out, creating two large plots (treated and untreated), each measuring 5x57metres. A week later, the transplant was carried out, keeping the seedlings - previously treated by dipping the roots in an aqueous suspension of mycorrhizal fungi and *Trichoderma artroviride* (Team mix 1.5 g/l)- separated from the untreated ones in order to create two blocks of seedlings (treated and untreated) for each plot (Figure 4). This led to the creation of 4 different theses, as follows: Thesis 1- Treated seedlings on treated soil; Thesis 2 - Treated seedlings on untreated soil; Thesis 3 - Untreated seedlings on treated soil; Thesis 4 - Untreated seedlings on untreated soil. The theses that included the treated seedlings were covered by a post-transplant application with the administration of Team mix (1Kg/ha) via fertigation. All thesis were continuously monitored with inspections every 15- 20 days. Only at harvest measurements on the productivity and health of the onions were carried out. The severity of the disease was measured with a standard scale from 0 to 5 where 0=refers to the absence of visible symptoms; 1= small alterations, less than 5%; 2=5-15% alteration; 3=15-25% alteration; 4 = 25-50% alteration; 5 = >50% alteration. The percent disease rate was calculated following a standard formula [9].

[X(f- v)/N x]100

(where: f = frequency of cases observed for each class, v = class value, N = number of observations and x = maximum class value of the scale adopted)

Figure 4: Setting up the trial to verify the intervention plan

Results

Monitoring and Phytosanitary Assessment for Correct Management of Adversities

During the various inspections, plants showing signs of stress were observed, including whitening, necrosis, wrinkling and sagging of the terminal parts of the leaves (Figure 5). Often, this decay was accompanied by damage to the roots and buried parts of the bulb, with decaying tissues taking on a pink color. Sometimes, pink rot affected only the outer skins of the bulb or only the roots. The isolation technique allowed us to ascertain the presence of three main pathogens: *Setophoma terrestris*, Fusarium spp. and *Colletotrichum circinans*. The first pathogen is the causal agent of Pink Root Rot and also affects many other horticultural plants, while the second is responsible for Fusariosis, which causes the rotting of bulbs in the field and in storage, and the third, *C. circinans,*is an agent of anthracnose. During the inspections, in the fields, in areas where the vegetation was compromised, plants with dwarfism, malformations and distortions of the stems and leaves, with discolored streaks on the stems and leaves were found. The alterations also affected the bulbs with the glassy and rippled external tunics, but the roots showed no obvious signs of alterations. In the laboratory, using a stereomicroscope, it was possible to associate these alterations with the presence of nematodes. In particular, the alterations only affected the bulbs and leaves, while the roots remained unchanged. *Ditylenchus dipsaci*, a nematode typical of the aerial parts of plants, has been associated with the symptoms observed.

Figure 5: Some kind of alterations detected on the onions while monitoring the farms in Margherita di Savoia

The technique allowed not only to confirm the presence of these nematodes, but also to quantify the number. In fact, the number of individuals present in 100g of soil varied from a minimum of 9 to a maximum of 16 specimens, confirming the infestation of the cultivations on the sandy beaches by these parasites.

In the Margherita di Savoia-Zapponeta area, due to the unfavorable climatic conditions for diseases of the aerial parts of onions, it was difficult to evaluate the reaction of the selections to attacks by pathogens. However, in the seed-bearing crops set up in Castelluccio dei Sauri, variability in the responses of the selections to downy mildew (Peronospora destructor) was observed (Figure 6). In general, the first infection occurred between 24 and 25 May 2021 for all selections, with the exception of the "Agostana" selection, for which the symptoms appeared earlier on 09 May 2021. Furthermore, considerable variability was found in the spread of the disease, with a percentage of infected plants detected on 28 May 2021 ranging from 20% for the "Early March" and "Late March" selections to 100% for the "Agostana" selection, while the others onion selections stand at 60% (Table 3).

Figure 6: Trials with seed bearing crops run in Castelluccio dei Sauri, on protected crops (non- woven tunnels) showing clear evidence of *Peronospora destructor*

Table 3: Appearance of first infections and percentage of infection of downy mildew (Peronospora destructor)

Selection	Appearance of the downy mildew	% of plants affected at the date 28/05/2021
1)Early March	25/05/2021	20%*
2)Late March	25/05/2021	20%*
3)Early March Del vecchio	25/05/2021	60%*
4)May flat	25/05/2021	60%*
5)June Frontino	24/05/2021	60%*
6)June Ruggiero	24/05/2021	60%*
7)Agostana	09/05/2021	100%*
*Percentage of plants with symptoms of downy mildew at the date 28/05/2021		

The phytosanitary status assessment activity led to sampling and isolation of onion seeds and external capsules, in order to determine whether the problem was due to infected or contaminated seeds. During the ripening of the inflorescences, random samplings of inflorescences were carried out, from which capsules were extracted and the seeds subjected to careful observations. From the seeds extracted from the different samples, 3 Petri dishes were prepared, each containing 5 seeds previously washed and disinfected like the other plant samples. The same procedure was also performed for the external capsules.

The inoculated Petri dishes were incubated at 25°C in dark conditions for 10-15 days. Subsequently, microscopic observations of the microbial flora that developed were carried out. The results of assessing the germination capacity of the seeds were obtained in four replicates of 100 seeds each, placing the seeds on filter paper soaked in sterile distilled water in glass Petri dishes, maintained at 20°C with 12 hours of light per day for 12 days. Germination was expressed as the percentage of seeds that germinated successfully [10].

Table 4: Assessment of the seed quality of the three selections of white onion of Margherita produced in Castelluccio dei Sauri

Onion selection	Germination%	Fungal species isolated from seed	Fungalspeciesisolated from capsules
MARZAIOLA	83,3	_	*Rizophus* spp*Alternaria* spp*Coniothyrium* spp
MAGGIAIOLA	93,3	_	*Colletotricum* spp*Alternaria* spp*Rizophus* spp
GIUGNAIOLA	86,6	_	*Humicola* spp*Coniothyrium* spp*Alternaria* spp*Penicillium* spp*Colletotricum* spp*Rizophus* spp

Table 5: Assessment of the seed quality of the three selections of white onion of Margherita grown in Margherita di Savoia

Onion selection	Germination%	Fungal speciesisolated from seed	Fungalspeciesisolatedfrom capsules
MARZAIOLA	86,6	_	*Fusarium* spp.*Alternaria* spp;micelio sterile
MAGGIAIOLA	66,6	_	*Fusarium* spp*Scopulariopsis* spp*Cladosporium* sppmiceli sterile
GIUGNAIOLA	80,0	*Alternaria* spp*Penicillium* spp	*Fusarium* spp*Alternaria* spp*Penicillium* spp*Chaetomium* spp

The results of the study of the quality of the seeds produced in the two listed environments, as reported in tables 4 and 5, indicate that all seeds do not show signs of contamination by fungal microorganisms considered pathogenic. However, a notable difference emerged in the composition of the fungal flora found in the external capsules of the two production locations. In particular, this difference resulted not only in a greater variety and richness in the composition, but also highlighted the presence of *Colletotricum* *spp*. in the "Maggiaiola" and "Giugniaiola" plants grown in the hinterland, and *Fusarium spp*. in all selections grown in the sandy areas of Margherita. Both isolated micromycetes are cited in the literature as pathogens, and therefore could be transmitted via contaminated seeds. From the same tables it emerges that there are no substantial differences regarding the germination parameter, although a certain variability can be noted between the three selections and the two production locations, with the seeds produced in purity appearing to be better.

Identification of the Intervention Strategy

After a careful phase of analysis of the existing problems, we proceeded to develop eco-compatible intervention strategies, as well as to define guidelines for the cultivation, in order to guide the sector's operators in the adoption of low environmental impact techniques.

The trials run revealed that the ineffectiveness of some treatments, such as Metam sodium and Brassicaceae flour, could be attributed to the use of already compromised propagation material. This underlines the importance of the sanitary status of the propagation material. In contrast, *Trichoderma harzianum* T-22 has proven to be quite effective in controlling fungal species of the Fusarium genus.

Even ASD, the subject of the second-year test, did not yield satisfactory results. The poor effectiveness is to be ascribed to the particular type of soil (sand) and the content of organic matter and nitrogen which play a fundamental role in the treatment of ASD, as these components promote the activity of the microorganisms involved in the process [11]. In fact, table 6, which indicates the level of nematode infestation in the soil, shows a limited presence in the control one without treatment (39 specimens per 100g of soil), while it reaches the maximum value (327 specimens) for the thesis with only grape pomace. This suggests that the exclusive supply of grape pomace increased the population of saprophytic nematodes. From the same table it emerges that the solarization of the thesis, combined with the organic soil improver, exerted a certain containment activity, leading to a lower charge (159 specimens).

This same trend was also found for the fungal load (colonies of *Fusarium* spp.), or rather, for the number of Colony Forming Units (CFU) per gram of soil, determined on a selective substrate for Fusarium. In fact, from table 7, the control thesis recorded lower values (12.47x103 CFU) of propagules of Fusarium spp. detected in the rhizosphere, highlighting higher values in the theses which included the addition of grape pomace, in particular for the thesis with grape pomace only (22.37x103 CFU), with a reduction (15.63x103 CFU) for thesis 1 (solarization +grape pomace). This not only confirms the effectiveness of solarization, but also demonstrates that some Fusarium species can adapt to a saprophytic life.

From table 8, which indicates the frequency of onion bulbs attacked by *Fusarium* spp., it emerges that the control thesis had 80% of infected bulbs, reducing to 60% for the thesis with pomace and further down to 40% for the thesis with solarization plus grape pomace. This data not only confirms the activity of solarization, but also demonstrates that the increase in the fungal population, in particular of *Fusarium* spp., is correlated to an increase in saprophytic forms favored by the enrichment of the organic substance, precisely because of the supply of pomace.

Table 6: Number of adult nematodes detected in the rhizosphere at the end of the fumigation trials run in 2022

Thesis	**N°/100g of soil**
Thesis	159
Thesis 2	327
Thesis 3	39

Table 7: Propagules of Fusarium spp. (UFC/g soil) detected in the rhizosphere at the end of t fumigation trials run in 2022

Thesis	Fusarium population (UFC/g)
Thesis 1	$15,63x10^3$
Thesis 2	$22,37x10^3$
Thesis 3	$12,47x10^3$

Table 8: Percentage of onion bulbs infected by Fusarium spp. at the end of the fumigation trials run in 2022

Thesis	Infected bulbs (%)
Thesis 1	40
Thesis 2	60
Thesis 3	80

Interesting results were obtained in trials run in the second year, using natural substances or microorganisms with repressive properties against nematodes and soil pathogenic fungi. In particular, the use of Brassicaceae extracts containing isothiocyanates and mycorrhizal plants with FMA has yielded promising results (Table 9 and Table 10).

Table 9: Diffusion (percentage of roots and bulbs) of the microbial flora detected

THESIS	Roots		Bulbs		
	Setophomaterrestris	Fusarium spp.	Fusarium spp.	Setophomaterrestris	Sclerotiumcepivorum
Thesis 1	46,6	13,3	26,6	43,3	6,6
Thesis 2	20	16,6	26,6	20	6,6
Thesis 3	40	12	20	23,3	6,6
Thesis 4	46,6	23,3	21,6	40	20

Table 10: Incidence (Index of McKinney) of the microbial flora detected

Thesis	Bulbs				
	Setophomaterrestris	Fusarium spp.	Fusarium spp.	Setophomate rrestris	Sclerotiumcepivorum
Thesis 1	12,5	5	6,6	13,3	2,5
Thesis 2	6,6	4,2	8,3	5,8	2,5
Thesis 3	10	6,6	7,5	5,8	1,6
Thesis 4	14	6,6	28,3	15	12,5

On the basis of this experimental evidence, an intervention plan was developed which provides for a first treatment seven days before transplanting and a second at the beginning of the development of the bulb, in fertigation with Isothiocyanate (rapeseed oil) - Senapoil - SKL at the rate of 5 kg per $1000m^2$. At the time of transplanting, the onion seedlings must be treated by immersing the roots in an aqueous solution with TEAM MIX (1.5g/l) and subsequently distributed through fertigation at a dose of 1Kg/ha.

The results of applying this protocol are very encouraging. From table 11, which reports the diffusion (percentage of symptomatic bulbs) and the incidence (Mc Kinney Index) of the alterations, it emerges that thesis 1 has diffusion values (25%) and incidence (7%) significantly lower than to all other theses. In particular, thesis 4 recorded diffusion values of 95% and incidence of 64%. It is important to note that this last thesis, which involves untreated seedlings on untreated soil, did not lead to any harvesting.

Table 11: Diffusion and Incidence of bulbs interested by alterations

Thesis	Class. 0 Absenceof symptoms	Class. 15%alteration	Class. 25-15%alteration	Class. 315-25%alteration	Class. 425-50%alteration	Class. 5>50%alteration	McKinney index(%)
Thesis 1	75	15	10	-	-	-	7
Thesis 2	40	15	15	10	5	-	19
Thesis 3	60	20	10	10	-	-	14
Thesis 4	5	10	25	10	20	30	64

Final Considerations

In conclusion, the production specialization, the use of the relevant inputs and the intensive cultivation techniques that characterize onion cultivation in the Margherita di Savoia countryside have caused a biological decline of the soil.

The activities carried out highlighted the phytosanitary problems affecting the "WHITE ONION OF MARGHERITA IGP" and identified the strategies to be adopted to achieve the objectives of the CIPOMAR project, namely, to improve the production system and product quality. The project provides practical suggestions and support to agricultural operators and technicians who operate in this specific area, complying with the legal provisions introduced by the National Action Plan for the sustainable use of plant protection products (PAN).

The interventions identified take into consideration not only the effectiveness but also the risk of contamination of surface water bodies resulting from the use of plant protection products. Since the onion cultivation area consists of small plots of shallow sandy soil between the sea and the Saline di Margherita, contamination can occur through three main routes: runoff, drift, and drainage. Under operational conditions, the priority risk is drift and drainage, therefore risk mitigation measures are required to reduce the input of plant protection products into surface waters.

This leads to the onion being repeatedly planted and grown in the same soil, generating an imbalance which is compensated by the excessive use of mineral fertilizers and pesticides. However, in the long run, this logic only leads to the insecurity of agricultural systems and the loss of soil.

The first rule for adequate sustainability is the maintenance of biodiversity, which can be achieved through rational agronomic practices such as crop rotation.

Inadequate crop rotation can lead the soil to develop fatigue, a phenomenon known for centuries in agriculture. It is based on the principle that all cultivated plants, even if to a different extent depending on the species, should not be repeated on the same soil.

Unlike conventional agriculture, organic agriculture constantly seeks sustainable production processes to respect the biological balance of the soil and increase fertility. With proper crop rotations, the problems and disadvantages mentioned above can be solved. Pest and fungal problems do not affect all plants equally; therefore, varying the type of crop is a preventive and, sometimes, curative solution. In the case of soil nematodes, indicative of an exhausted soil, Brassicaceae can be used (as cover crop), which have a natural biocidal activity, or crops can be varied to reduce the nematode population, choosing plants with a low reproduction rate for the nematode species present in the area. [12].

A period of set-aside should also be considered.

It's not a bad thing for the earth to rest, on the contrary, one can take advantage of the break to introduce green fertilization (or green manure with brassicas).

It is also important to consider resting periods for the soil, during which green fertilizers can be applied (such as green manure with Brassicaceae). In this way, the cycle becomes virtuous, and it is not necessary to use synthetic chemical pesticides. Observations and literature indicate that the use of natural substances with biological activity against soil pathogens is effective for sustainable fertility management.

The correct production practice is based on the principles of integrated defense, which implies the adoption of measures to reduce parasite attacks, considering the disease triangle (host-pathogen- environment). The management of adversities in the cultivation of the "White Onion of Margherita" must include preventive measures such as crop rotation, the use of healthy seeds or seedlings and the reduction of fertilization, following an integrated pest management perspective. The adoption of genetic means is still being evaluated as the project is ongoing and the response of plants to different pathogens in the absence of phytogenic interventions is being studied, in order to identify resistant and uniform populations.

The creation of conditions that respect the natural balance of the soil, typical of organic cultivation practices, represents the preliminary and preventive phase in the defense of crops from harmful agents of a biotic and abiotic nature. Cultivating local ecotypes adapted to specific soil and climate conditions, together with the supply of organic substance in fertilization and other cultivation techniques, helps plants to become less susceptible to infections and damage from harmful agents. Furthermore, the propagation material must be free of pathogens, as demonstrated by the first soil geo-disinfestation trials carried out in the first year.

As regards open field crops, currently the impossibility of directly controlling some harmful agents such as phytopathogenic nematodes and Fusarium makes it necessary to adopt long-term crop rotations, at least 5 years. However, in the Margherita di Savoia countryside, the small size of the parcels makes such an extensive cycle impracticable. Consequently, the use of traditional crop rotations is difficult in intensive cropping systems. Another promising option is microbiological control. As demonstrated by studies conducted [13], Fusarium can be managed using various biological control agents, including *Trichoderma gamsii, Fusarium oxysporum IF23, Streptomyces griseoviridis* and *Bacillus subtilis*. Alternatively, it is possible to use biofumigants for soil disinfection, especially considering the presence of nematodes. An interesting option in organic farming is the use of plants from the Brassicaceae family as cover crop. These plants, thanks to their natural biocidal activity, can control nematode infestations and improve soil health. Brassicaceae are known to be soil-improving plants and contain a natural defense system based on glucosinolates and myrosinase, which releases allelochemicals such as isothiocyanates and nitriles. These compounds have biocidal activity against nematodes and fungi present in the soil [14].

To maximize effectiveness, it is advisable to chop and completely incorporate the Brassicaceae plants (to a depth of 15-20 cm) using the green manure technique, or use them in the form of powder or pellets, especially in intensive cultivation systems [15, 16].

For the containment of soil pathogens and parasites, it seems logical to focus more on techniques that use mild means capable of preserving the biocenosis of the soil. In fact, through the application of the solarization technique in combination with the use of grape pomace, it was deemed to implement a bio solarization process capable of enhancing the effect of solar heat. It has actually been found that the solarization technique alone can constitute an adequate method to weaken pathogenic fungi and act effectively against nematodes.

Probably, with the identification of more appropriate soil amendments, the effects can be improved, as demonstrated by other studies [3-7]. Organic soil improvers with a high nitrogen content, such as compost and manure, have been successfully used to contain various soil-borne pathogens [3, 7]. Furthermore, the need to incorporate large quantities of soil improvers in the soil is also an aspect to consider both in terms of supply and distribution logistics and possible undesirable effects, such as the accumulation of nitrates in the soil [17].

From preliminary observations, the importance of using healthy propagation material emerges since both *Colletotrichum* and *Fusarium*, which infect capsule tissues, can be transmitted through contaminated seeds. This is consistent with what is reported in the literature, where it is stated that various soil pathogens, including *Fusarium oxysporum*, can be transported and spread through infected or contaminated seeds [18]. It is therefore crucial to adopt techniques that reduce the risk of parasite transmission through the propagation material. Seed treatment with suitable fungicides is effective, but unfortunately there are few chemicals authorized for this purpose. Therefore, there is a growing interest in physical methods and organic and natural products [19, 20]. In the case of onion seeds, it has been found that heat treatment in water (immersion of the seeds in hot water at 57°C for 5 minutes) can be effective.

The goal of developing disease prevention and control methodologies without the use of chemicals can be achieved by exploiting genetic resistance. Therefore, it is important to evaluate the susceptibility of White Margherita Onion selections to biotic and abiotic diseases. However, the adverse climatic conditions for the development of onion aerial diseases have made it difficult to evaluate the behavior of selections with respect to pathogen attacks, although variability between selections in downy mildew (*Peronospora destructor*) infestation was observed in seed-bearing crops at Castelluccio dei Sauri, suggesting that onion selections may react differently to other diseases.

Furthermore, although crop monitoring has not revealed any particular phytosanitary problems linked to climatic conditions, it is important to protect plants from possible diseases when the risk becomes excessively high, following eco-sustainable standards for phytosanitary defense and control of crop weeds of the crops of the Puglia region.

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