SERBIA ACCELERATING INNOVATION AND GROWTH ENTREPRENEURSHIP (SAIGE) PROJECT

Program PROMIS

ENVIRONMENTAL AND SOCIAL MANAGEMENT PLAN (ESMP)

A "vaccine" for black rot – biocontrol of *Xanthomonas campestris* pv. *campestris* on autochthonous cabbage cultivar Futoški using plant-associated beneficial bacteria (XanthoSTOP)

DRAFT DOCUMENT

Belgrade,

18/12/2023

ABBREVIATIONS AND ACRONYMS

EPPO – European and Mediterranean Plant Protection Organization

ESMF – Environmental Social Management Framework

ESMP – Environmental and Social Management Plan

GRAS – generally recognized as safe

HPLC – High Performance Liquid Chromatography

IFVCNS – Institute of Field and Vegetable Crops, Novi Sad

IMSI – University of Belgrade – Institute for Multidisciplinary Research

NITRA – Ministry of Science, Technological Development

PI – Principal Investigator

PIU – Project Implementation Unit

SAIGE – Serbia Accelerating Innovation And Growth Entrepreneurship

SF – Science Fund

SRO – Scientific and Research Organization

WP – Work Package

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EXECUTIVE SUMMARY

Environmental and Social Management Plan (ESMP) draft has been prepared for the PROMIS Program, funded by the Science Fund of the Republic of Serbia (SF). The main goals of the PROMIS Program are to support young researchers with exceptional ideas that have a positive impact on society and the economy, to strengthen their professional capacities, provide them with experience in project management, and encourage them to apply for other research and development projects at the national and international level. The draft ESMP document for the project entitled "A "vaccine" for black rot – biocontrol of *Xanthomonas campestris* pv. *campestris* on autochthonous cabbage cultivar Futoški using plant-associated beneficial bacteria" (hereinafter: XanthoSTOP), was prepared in accordance with Environmental and Social Management Framework (ESMF) for the Serbia Accelerating Innovation and Growth Entrepreneurship (SAIGE) Project. According to the environmental and social checklist screening, which was carried out by Environmental, Social, and Ethics Experts during the project evaluation, XanthoSTOP was ranked as a project with low ethical risk and moderate risk with regard to the environment and society.

To mitigate negative effects of pesticides on food quality, the environment, and human health, it is important to prioritize the development and implementation of more sustainable crop management practices that rely less on synthetic chemicals. Therefore, the European Commission provided a protective plan within the European Green Deal "Farm to Fork Strategy", aiming to achieve a 50% reduction in the use of chemical pesticides by 2030. Consequently, the inclusion of microbial-based bactericides (especially those based on plant-associated bacteria) into disease management practices is becoming an integral part of modern agriculture.

In alignment with the mentioned European Commission's Green Deal and Serbian Strategy of Smart Specialization, the main objective of the XanthoSTOP project is to develop an environmentally friendly substitute for chemical pesticides to effectively manage black rot caused by *Xanthomonas campestris* pv. *campestris* on autochthonous cabbage cultivar Futoški. This environmentally friendly approach is based on reintroducing of "good" bacteria that are already part of the microbiome of cabbage cv. Futoški, to combat the growth of *X. campestris* pv. *campestris* which will result in decreased disease intensity (black rot) and reduced need for chemicals. By intensifying the use of bio-pesticides, this project will help to mitigate the potential risks associated with pesticide exposure and will minimize the likelihood of experiencing adverse health effects. The project plays a crucial role in preserving soil health and preventing water system contamination with chemical residues and highlights its commitment to sustainable agriculture, enhanced food quality, and a healthier environment. Consequently, the project will contribute to biodiversity conservation in the agricultural ecosystem, with a particular focus on preserving the indigenous microbial diversity of the autochthonous cabbage cultivar Futoški.

The ESMP document outlines precise actions for monitoring all potentially adverse environmental and social impacts of the XanthoSTOP project and for mitigating them (eliminating or reducing them to acceptable levels) in compliance with relevant local policies and legislation, as well as the World Bank requirements. The document is dedicated to ensuring compliance of the Scientific Research Organization (SRO) involved in the project (University of Belgrade – Institute for Multidisciplinary Research, IMSI) with the health and safety regulations, and environmental protection throughout the project implementation, with a particular focus on field experiments.

LEGAL AND ADMINISTRATIVE FRAMEWORK

Relevant Institutions

Relevant institutions involved in the realisation of the XanthoSTOP project are University of Belgrade – Institute for Multidisciplinary Research (IMSI) and the Ministry of Science, Technological Development (NITRA). NITRA is responsible for the implementation of the SAIGE project.

Existing Serbian legislation

All field work, sampling procedures, laboratory work, and waste management of the XanthoSTOP project will be in accordance with the relevant laws, regulations, and/or management strategies of the Republic of Serbia, including specific rulebooks:

- Law on Science and Research of the Republic of Serbia ("Official Gazette of RS", 49/2019);
- Law on science ("Official Gazette of RS", 116/2007, 88/2009, 88/2009, 104/2009, 10/2015, 36/2018);
- Law on environmental protection ("Official Gazette of RS", 135/2004, 36/2009, 72/2009, 43/2011, 14/2016, 76/2018, 95/2018);
- Law on soil protection ("Official Gazette of RS", 112/2015);
- Law on agriculture land ("Official Gazette of RS", 62/2006, 65/2008, 41/2009, 112/2015, 80/2017, 95/2018);
- The law about plant protection products ("Official Gazette of RS", 41/2009, 17/2019);
- Law on special powers for the purpose of efficient protection of intellectual property rights ("Official Gazette of RS", 46/2006, 104/2009, 129/2021);
- Labour law ("Official Gazette of RS", 4/2005, 61/2005, 54/2009, 32/2013, 75/2014, 13/2017, 113/2017, 95/2018);
- Law on Occupational Health and Safety ("Official Gazette of RS", 35/2023);
- Law on disaster risk reduction and emergency management ("Official Gazette of RS", 87/2018);
- Law on fire protection ("Official Gazette of RS", 111/2009, 20/2015, 87/2018, 87/2018);

- Law on waste management ("Official Gazette of RS", 36/2009, 88/2010, 14/2016, 95/2018);
- Law on chemicals ("Official Gazette of RS", 36/2009, 88/2010, 92/2011, 93/2012, 25/2015);
- Law on free access to information of public importance ("Official Gazette of RS", 120/2004, 54/2007, 104/2009, 36/2010, 105/2021);
- Law on noise protection ("Official Gazette of RS", 96/2021);
- Law on disaster risk reduction and emergency management ("Official Gazette of RS", 87/2018);
- Law on protection of personal data ("Official Gazette of RS", No. 87/2018)
- The law on data confidentiality ("Official Gazette of RS", No. 104/2009)
- Law of gender equality ("Official Gazette of RS", No. 52/2021)
- Rulebook on preventive measures for safe and healthy work at the workplace ("Official Gazette of RS", 21/2009, 1/2019);
- Rulebook on personal protective equipment ("Official Gazette of RS", 23/2020);
- Rulebook on the provision of signs for safety or health at work ("Official Gazette of RS", 95/2010, 108/2017);
- Rulebook on records in the field of safety and health at work ("Official Gazette of RS", 62/2007, 102/2015);
- Rulebook on the providing of first aid, the type of means and equipment that must be provided at the workplace, the method and deadlines for training employees to provide first aid ("Official Gazette of RS", 109/2016);
- Rulebook on preventive measures for safe and healthy work when exposed to chemical substances ("Official Gazette of RS", 106/2009, 117/2017, 107/2021);
- Rulebook on preventive measures for safe and healthy work when using work equipment ("Official Gazette of RS", 23/2009, 123/2012, 102/2015,101/2018);
- Rulebook on records in the field of safety and health at work ("Official Gazette of RS", 62/2007, 102/2015);
- Rulebook on the procedure for inspecting and checking work equipment and testing working environment conditions ("Official Gazette of RS", 94/2006, 108/2006, 114/2014 102/2015);
- Rulebook on categories, testing and classification of waste ("Official Gazette of RS", 56/10, 93/2019);
- Law on chemicals ("Official Gazette of RS", 36/2009, 88/2010, 92/2011, 93/2012, 25/2015);
- Rulebook on how to keep records on chemicals ("Official Gazette of RS", 31/2011);
- Rulebook on the register of chemicals ("Official Gazette of RS", 16/2016, 6/2017, 117/2017);
- Rulebook on chemical advisors and the conditions that must be met by a legal entity or entrepreneur who conducts training and knowledge testing of chemical advisors ("Official Gazette of RS", 13/11, 28/11, 47/12),

- Rulebook on the manner of storage, packaging and labelling of hazardous waste ("Official Gazette of RS", 92/2010, 77/2021);
- Strategy of scientific and technological development of the Republic of Serbia for the period 2021-2025 ("Official Gazette of RS", 10/2021)

Relevant specific documents for IMSI

The integration of IMSI into the SAIGE project has prompted an update of Rulebooks regarding general safety and health at work that are expected to be finalized in the forthcoming period. The currently existing regulations at IMSI, related to the project theme are listed below:

- Rulebook on safety and health at work (2008, IMSI). The development of a new rulebook on safety and health at work at IMSI is currently in progress and is scheduled to be established at the beginning of 2024.
- Rulebook on the use and maintenance of equipment at the Institute for Multidisciplinary Research (2012, IMSI). The development of a new rulebook at IMSI is currently in progress and is scheduled to be established at the beginning of 2024.
- Rulebook for the safe work in labs at the Life Sciences Department (2023, IMSI)
- Rulebook for the safe work in molecular biology lab (2022, IMSI),
- Guidebook on the safe use of chemicals in laboratories (2022, IMSI),
- Decision on appointing a person responsible for waste management (2023, IMSI),
- Report on the theoretical and practical training of the knowledge check in the field of Fire Protection for all employees (2015, IMSI). The preparation of a new rulebook for fire protection at IMSI is currently in progress and is scheduled to be established at the beginning of 2024. Theoretical and practical training will also be held at the beginning of the upcoming year.
- The implementation of effective safety protocols for fieldwork will be guaranteed through the development of precise "Guidelines to facilitate the work of researchers in the field".

PROJECT DESCRIPTION

INSTITUTIONAL AND ADMINISTRATIVE PART	
Country	Serbia
Project	Serbia accelerating innovation and growth entrepreneurship (SAIGE) project
Sub-component	Science Fund of the Republic of Serbia

Program	PROMIS
Subprogram	Natural Sciences
Project title	A "vaccine" for black rot – biocontrol of *Xanthomonas campestris* pv. *campestris* on autochthonous cabbage cultivar Futoški using plant-associated beneficial bacteria
Acronym	XanthoSTOP
PI email address	aleksandra.jelusic@imsi.rs
Participating Scientific and Research Organization (SRO)	University of Belgrade – Institute for Multidisciplinary Research
The duration of the project	24 months
Number of researchers	5 (PI+4 researchers)

The project XanthoSTOP aims to develop an environmentally friendly alternative to chemical pesticides, commonly used for black rot control, by harnessing beneficial properties of plant-associated bacteria isolated from cabbage cv. Futoški (appellation of origin, The Intellectual Property Office of the Republic of Serbia, https://www.zis.gov.rs/en/rights/indications-of-geographical-origin/statistics/). Plant pathogenic bacterium *X. campestris* pv. *campestris*, the causal agent of black rot disease, was selected as a target pathogen for this project due to its global economic importance in affecting the yield, growth, and quality of cabbage and other hosts within the Brassicaceae family (broccoli, cauliflower, collard greens, kale, kohlrabi, oilseed rape, etc.). *Xanthomonas campestris* pv. *campestris* is ubiquitous in Serbia and worldwide and occurs every year in fields growing Brassicaceae. According to the protective measures against plant pests, provided by the European Commission (EU_2019_2072) and also the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, *X. campestris* pv. *campestris* is not designated as a quarantine organism. Various agrotechnical (e.g. rotation with non-host/unrelated crops), physical (e.g. seed treatment with hot water), and chemical (e.g. copper-based bactericides) disease management strategies are nowadays being used for managing black rot. To mitigate negative effects of pesticides use, it is of utmost importance to prioritize the development and implementation of more sustainable crop management practices that rely less on synthetic chemicals. Therefore, within the XanthoSTOP project, we will isolate indigenous, biocontrol strains from the phyllosphere and rhizosphere of cabbage cv. Futoški and evaluate their efficacy in suppressing the growth of *X. campestris* pv. *campestris*, through *in vitro* and *in vivo* (greenhouse and field) assays. The criteria for selecting the most potent biocontrol candidate(s) will include evaluating indigenous bacterial strains for properties related to biocontrol, human safety, resilience to

environmental factors, suitability for large-scale production, and affiliation to Biosafety Risk Group 1 (biological agents which are unlikely to cause disease in an individual) and GRAS (generally recognized as safe) group of microorganisms. Considering all of the above mentioned, handling these bacteria does not present danger either for the team members that are directly involved in working with them, or for the environment.

PROJECT LOCATIONS

Project implementation will take place in different locations, i.e.

Experiments in the field

1. Collection of the plant (leaves) and soil (rhizosphere) material for bacterial isolation will be performed at two privately owned agricultural fields cultivating cabbage cv. Futoški. Both fields are located in Futog (field I: 45.248448, 19.715581; field II: 45.2515990, 19.7236130). "Land use agreement" will be signed with the owner of the agricultural fields before the planned project activities begin. To ensure following all the rules signed by the agreement, field's owner will be present in the field during the realization of this project activity. Both fields are surrounded by other fields, also dedicated to agricultural activities, ensuring their isolation from urban neighbourhoods, major roads, waterways, and similar entities. These fields are located near several civil households and therefore will only be used for sampling the soil and cabbage leaves to isolate pathogenic and bacterial biocontrol candidates for project experiments.

In accordance with the planned project activities, from each field, we will sample per three leaves from the five randomly selected cabbage plants (with and without black rot symptoms) and around 5-10 g of their corresponding rhizosphere (from a 5 cm depth). Collected samples will be placed in sterile sampling bags (leaves) and falcons (rhizosphere) and transported to the laboratories of IMSI (Department of Life Sciences) for bacterial isolation. Sampling will be performed following the standard safety microbiological protocol for field work (nitrile gloves, sterilization of tools between sampling points, etc.). Team members in charge for sampling are familiar with all of the risks of working in the field. For any accidental events that could occur during their stay in the field [e.g. allergic reaction (insect bite, pollen, etc.), injury from a fall, a cut, etc.] a first aid kit will always be close at hand and transport will be parked in close proximity to the sampling location, allowing fast transfer of injured person to the nearest ambulance. To eliminate any potential risks from weather-related extreme events (e.g. extreme temperatures, thunderstorms, etc.) during sampling (August/September), we will carefully plan this project sub-activity in advance. This planning will involve thorough consideration of the weather forecast, ensuring that all research activities are conducted on days with favourable weather conditions.

2. Field trial with biocontrol candidate(s) will be performed at another private agricultural field (45.2581580, 19.7056740), also located in Futog. "Land use agreement" will be signed with the owner of the field before the planned project activities begin. Unlike the two aforementioned fields (fields I and II), this field is totally isolated from households. Therefore, it has been designated as a perfect spot for conducting biocontrol field experiments that involve artificial inoculation of cabbage plants with *X. campestris* pv. *campestris*, treatments with biocontrol candidate(s) and their comparing to chemical ones (commercially available), most commonly used for black rot control. In order to ensure the implementation of all necessary measures to prevent the transmission (via raindrops, wind, etc.) and persistence (in the soil) of *X. campestris* pv. *campestris* during and after the experiments, we have chosen a field where tomato plants (from the Solanaceae family), which are non-hosts for *X. campestris* pv. *campestris*, are cultivated. Furthermore, plants from the Brassicaceae family are not grown in any of the agricultural fields located in the immediate vicinity of our field of interest. In compliance with all planned protection measures, treatment of cabbage cv. Futoški plants with selected *X. campestris* pv. *campestris* strain poses no risk to the surrounding environment.

Furthermore, the application of biocontrol treatments will not cause any damage to the individuals and/or the environment, since the key criteria for the selection of bacterial biocontrol candidate(s) among all isolated autochthonous strains will be their affiliation to the biosafety Risk group 1 and GRAS group of microorganisms.

Control treatments of cabbage cv. Futoški plants with some commercially available pesticide for black rot control (e.g. copper-based pesticide) will serve as standard, for evaluation of the efficacy of the applied biocontrol treatments. Given the number of the small plants treated (up to 30 cabbage plants), this treatment is not anticipated to result in any adverse effects on the environment or individuals.

The whole experiment (sowing, application of treatments, monitoring, and harvesting) will be conducted using suitable equipment (appropriate clothing, protective mask, safety glasses, gloves, etc.) and following a grace period for pesticide use (according to the producer's instructions). All activities will be carried out with the support of the field owner, following the previously established "Land use agreement".

Experiments in the greenhouse

Greenhouse experiments related to evaluating the pathogenicity and virulence of *X. campestris* pv. *campestris* strains and the efficiency of biocontrol candidates *in vivo* on cabbage cv. Futoški plants, will be performed at the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia (IFVCNS), Department for Oil Crops (45.328817, 19.827944). Experiments will be performed with the assistance of colleagues from the IFVCNS, according to the previously signed contract on scientific and technical cooperation (in the process of signing).

The experiments will be performed under controlled conditions in the greenhouse. Therefore, they will not have any direct effect on the environment (air, soil, or water quality). Plants for the experiments will be grown in pots filled with previously sterilized soil. Control treatments for the biocontrol experiment will imply spraying of cabbage cv. Futoški plants with some commercially available pesticide for black rot control (e.g. copper-based pesticide), that will serve as standard for evaluation of the efficacy of our biocontrol treatments. During the spraying of plants with the selected pesticide, strict safety regulations will be followed in terms of the used protective equipment (appropriate clothing, protective mask, safety glasses, gloves, etc.). Upon finishing the experiments, plants and soil that were treated with *X. campestris* pv. *campestris* and/or biocontrol candidate(s) will be disposed to autoclavable biohazard bags, heat-sterilized (autoclave) and discarded following strict protocols.

Experiments in the laboratory

All laboratory experiments will be performed at the Department of Life Sciences of the University of Belgrade – Institute for Multidisciplinary Research, IMSI (44.81707, 20.48694). Laboratory experiments related to isolation of the *X. campestris* pv. *campestris* and the potential biocontrol strains from the collected plant and soil material and their cultivation will be performed according to microbiological protocols. These activities will be performed under controlled conditions in the laboratory, without producing any direct effect on the environment (air, soil, or water quality). Plant pathogenic bacterium *X. campestris* pv. *campestris* is ubiquitous and occurs every year on Brassicaceae plants in Serbia and worldwide. According to the protective measures against pests of plants provided by the European Commission (EU_2019_2072), but also the Ministry of Agriculture Forestry and Water Management of the Republic of Serbia, *X. campestris* pv. *campestris* is not designated as a quarantine organism. Therefore, handling of this pathogen is not subject of specific regulatory measures but rather follows conventional microbiological practices.

Isolation and handling of the autochthonous bacterial strains with biocontrol properties pose no risk to the researchers or the environment. The criteria for selecting biocontrol candidate(s) will include their evaluation for properties related to biocontrol, human safety, resilience to environmental factors, suitability for large-scale production, and affiliation to Biosafety Risk Group 1 and GRAS group of microorganisms.

Team members are well-trained for work in laboratory and aware of strict safety guidelines for work in labs at the Life Sciences Department (2023, IMSI). All microbiological waste and microbiologically contaminated plastic materials (tips, tubes, Petri dishes) generated during experiments will be heat-inactivated (autoclaved) before being discarded in labelled biohazard bags, following the waste management plans and rulebooks at SRO.

Experiments related to work in the laboratory for molecular biology (DNA isolation, identification of the *X. campestris* pv. *campestris* and biocontrol candidates; detection of genes encoding the production of antimicrobial secondary metabolites) will be performed in accordance with the Rulebook for the safe work in molecular biology lab (2022, IMSI).

Solutions containing midori green dye for DNA staining (agarose gels) will be discarded in hazardous waste bags and picked up by the waste management company.

Disposal of waste resulting from biochemical and analytical experiments, such as assessing enzyme activities and detecting metabolites like flavonoids, glucosinolates, and ascorbate, will adhere to waste management plans outlined in the IMSI. The chemical waste produced during the project will be in a small-scale range, amounting to several kilograms. The waste generated during these experiments consists of chemicals, commonly used in biochemical and analytical laboratories (e.g. ethanol, acetone, methanol, acetic acid, phosphate buffer, sodium chloride, ethylenediaminetetraacetic acid, hydrochloric acid, chloroform, acetonitrile, sodium hydroxide, ascorbic acid, etc.). The utilization and proper disposal of all chemicals employed throughout the project will adhere to the principles of good laboratory practice.

ASSESSMENT OF THE POTENTIAL ENVIRONMENTAL AND SOCIAL IMPACTS OF SPECIFIC TASKS WITHIN THE PROJECT

Potential impact on sampling sites

Sampling of soil and cabbage leaves for isolation of bacterial strains does not impact the local ecosystem and biodiversity in any way. The experiment requires approximately 5-10 grams of soil in five replicates, equalling 25-50 grams from one field in total. Soil sampling will be conducted manually using a small metal spatula without any mechanization. Given that only small amount will be sampled, there is no possibility that this activity could affect the soil composition. The collection of plant material (three leaves from five randomly selected cabbage plants with and without symptoms of black rot) will also follow the standard protocol for sampling plant material, ensuring it has no negative impact on the environment. This activity doesn't generate noise or disturb citizens in any way.

Potential impact of generated waste

Fieldwork does not produce hazardous infectious or chemical waste. However, all waste generated during fieldwork, including papers, paper boxes, plastics, and other waste, will be collected afterward and properly disposed.

Non-biological waste, including plastic consumables and paper, will be managed according to the waste management plans at SRO. Potentially microbiologically contaminated plastic materials (tips, tubes, Petri dishes), along with liquid waste like cultivation media will be heat-inactivated (autoclaved) before being discarded in labelled biohazard bags, following the waste management plans and rulebooks at SRO.

Hazardous chemical waste, including organic acids, strong inorganic acids and flammable or corrosive substances, shall be stored safely in designated containers and flasks that are protected against leakage and spillage. These containers will be clearly labelled for easy identification. The labelled waste will then be stored in a specially designated area of the SRO not to pollute the environment and will be regularly removed by specialized companies in

charge of chemical waste disposal. Within the SRO, there is a person responsible for waste management protocols. Throughout the implementation of the project, the amount of hazardous and non-hazardous waste will remain minimal, amounting to only several kilograms over the entire duration of the project.

Potential impact on the health and safety of the project team

Ensuring the safety of team members is a top priority at all times. Each team member is a employee of the SRO and must comply with national health and safety laws and other relevant legal documents relating to safety in the workplace.

Regarding field activities, planned tasks are not expected to jeopardize the safety of project team members. The safety of participants could only be endangered by unforeseen events such as insect or snake bites, accidental injuries or falls. In the event of unforeseen situations during the implementation of experiments in the field, a minimum of three team members will partake in the activities, with at least two of them holding valid driver's licenses. Therefore, transportation to the nearest ambulance will be organized immediately. In addition, the team members will have a portable first aid kit and suitable personal protective equipment (e.g. rubber boots, gloves, hats, adequate clothing, etc.). In order to minimize possible weather-related risks, the field experiments will be meticulously planned taking into account the weather forecast. Safety measures during collecting and handling of samples, as well as biocontrol treatment (like using sterile gloves, safety glasses, and sterilization of tools between sampling points) will be carried out by team members who are skilled, experienced, and also familiar with regulations regarding code practice during fieldwork.

As far as laboratory work is concerned, all researchers are trained to work safely following the principles of good laboratory practice. During the experimental work in the laboratory, researches will wear lab coats, nitrile gloves and protective glasses, appropriate footwear, long hair will be tied back, etc. The laboratory work adheres to strict SRO protocols to ensure safety and sterility. Possible consequences on the health and safety of project team members may only occur from accident situations such as falls, burns or cuts. In this case, first aid will be administered and the injured person transported to the nearest ambulance.

Community health and safety

The implementation of the project does not have negative impact on the health and safety of the community. Field experiments will not produce any noise or disturb local inhabitants. Treating plants with biocontrol and pathogenic bacterial strains, naturally isolated from those plants, does not present a threat to the air, soil, or surface waters. Moreover, the application of commercially available chemical products for disease control (as anticipated by the project) will strictly adhere to the Law on plant protection products of the Republic of Serbia and will be done in accordance with internal procedures. Adhering to laws and regulations concerning occupational safety, waste disposal procedures, fire safety, and responsible laboratory

practices is essential in averting eventual negative impacts of project activities on the health and welfare of the community.

Potential socio-economic impact

The project does not have any negative socio-economic impact; on the contrary, a significant positive potential impact is projected and expected. The results obtained will offer an environmentally friendly solution for the control of black rot in cabbage and thus contribute to sustainable agriculture by reducing the use of pesticides in disease control. By minimizing the reliance on chemical pesticides, XanthoSTOP project plays an important role in preserving soil health and preventing water system contamination with chemical residues. This commitment to reducing chemical inputs promotes the overall environmental sustainability of agricultural systems.

The results of the project have the potential to encourage cooperation between scientific community and the agricultural industry by facilitating the exchange of knowledge, transfer of technology, and the consideration of potential economic partnerships. The results of the XanthoSTOP project are also expected to have an impact on the scientific community interested in crop protection research, as they provide comprehensive insights into the interactions between biocontrol candidates, plants and pathogens in general.

Primary beneficiaries (e.g. cabbage growers) will be provided with a safer and eco-friendly alternative for black rot management which implies decreased chemical exposure during spraying, lower costs associated with expensive pesticides, and the ability to market high-quality organic products. Beyond producers, consumers will also benefit from access to healthier and organic food choices.

SUMMARY OF ENVIRONMENTAL AND SOCIAL IMPACT

All potential negative environmental impacts that could occur during the implementation of the research project titled "A "vaccine" for black rot – biocontrol of *Xanthomonas campestris* pv. *campestris* on autochthonous cabbage cultivar Futoški using plant-associated beneficial bacteria" (XanthoSTOP) are listed below, together with the intensity of their actions.

Table 1. Review of the impact on the environment that are predicted for the duration of the project

INFLUENCE	SIGNIFICANCE	COMMENT
Impacts on land use and settlements	Does not exist	During the realization of the project, there will be no expropriation of land
Ground and surface water	Low	The recipient experiences negligible consequences due to the low water intake through the drainage system.

Table 1. Review of the impact on the environment that are predicted for the duration of the project

INFLUENCE	SIGNIFICANCE	COMMENT
Air quality	Does not exist	Impact is negligible.
Flora and fauna (protected areas and species)	Low	Sampling locations are not within protected areas with endangered and protected species
Noise	Does not exist	Impact is negligible.
Soil management	Low	With the application of appropriate measures of waste management
Management of Waste	Low	Ensured in accordance with the existing waste management plan.
Management of hazardous materials, including hazardous waste	Low	Ensured in accordance with the existing waste management plan.
Medical waste management	Does not exist	Impact is negligible.
Working in the laboratory including Life and Fire Safety	Moderate	Safety of the team members will be ensured by following strict procedures given by SRO in the Rulebook for the safe work in lab. With the application of appropriate protective equipment, and personal training, the impact is low.
Working in the field	Moderate	Measures for safety during fieldwork will be applied in accordance with updated protocols. During the fieldwork, members of the project team could be exposed to the bites of insects and animals, or to the extreme weather conditions (high temperatures and thunderstorms). With the application of appropriate protective equipment and training of personal the impact is low.
Safe management of chemicals, biohazards and hazardous materials	Low	Ensured through management of chemicals, biohazards and hazardous materials – management plan will be prepared and implemented
Handling of gases under pressure (Health & Safety	Does not exist	During the realization of the project, there will be no handling of gases under

Table 1. Review of the impact on the environment that are predicted for the duration of the project

INFLUENCE	SIGNIFICANCE	COMMENT
at work and prevention of accidents)		pressure.
Health & Safety of the local populations (Field activities)	Low	Throughout the implementation of the projects field activities, there will be no negative impacts on the health and safety of the local population.
Use of chemicals	Low	All researchers in the research laboratory are familiar with safety procedures related to working with chemicals and all Safety Data Sheets for Chemicals.
Cumulative impacts	Moderate	Members of the project team could be exposed to potentially accidental situations during field and laboratory work.

18/12/2023
ESMP prepared by:
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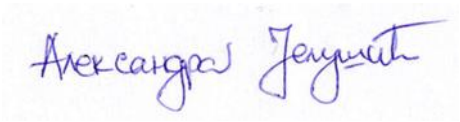

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MITIGATION PLAN

Phase	Issue	Mitigating Measure	Cost of Mitigation (If Substantial)	Responsibility	Supervision
Project preparation					
Project preparation	Land use agreement, necessary for access to all of the planned sampling locations and conducting biocontrol experiments will be prepared before beginning of the activities. Due to unforeseen circumstances field(s) location might become unavailable.	New land use agreements will be prepared if required.	None anticipated for preparing land use agreement; possible costs will be covered by IMSI or project overhead	PI, IMSI	PIU/SF/IMSI
Project preparation	Lack of specific code of practice for fieldwork in accordance with planned activities within XanthoSTOP.	Creation of appropriate documents before the start of the activities on the field	None	IMSI, PI	PIU/SF/IMSI
Project preparation	Agreements on scientific and technical cooperation with IFVCNS for greenhouse experiments. Due to unforeseen circumstances greenhouse might become unavailable.	Creation of appropriate agreements. New agreement on scientific and technical cooperation will be prepared if required.	None	PI, IMSI	PIU/SF/IMSI
Project preparation	Life and fire safety procedures in laboratory	All team members are familiar with the current Evacuation Plan and Protection and Rescue Plan, with the dangers of fire and fire protection measures and are trained in handling fire	None	IMSI	SF/PIU

		extinguishers, hydrants and other devices used for extinguishing fires by the Law, as well as with the "Instructions for action in case of fire".			
Project implementation					
Project implementation	Exposure of team members to potentially accidental situations during the fieldwork (bites of insects, animals, allergies)	Sampling performed by experienced and previously trained team members, in accordance with specific code of practice for fieldwork given by IMSI.	None	PI, IMSI	PIU/SF/IMSI
Project implementation	Weather-related extreme events during field work	Thoroughly planning the fieldwork in advance, considering the weather forecast, will be undertaken to ensure that the fieldwork is conducted under suitable weather conditions.	None	PI, IMSI	PIU/SF/IMSI
Project implementation	Leaving plastic and paper waste during sampling in the field.	All plastic and paper waste will be collected and properly disposed.	None	PI, IMSI	PIU/SF/IMSI
Project implementation	Emissions by vehicle used for field work.	One car will be used to visit multiple fields. Field trips will be carefully planned to optimize fuel consumption.	None	PI, IMSI	PIU/SF/IMSI
Project implementation	Team members safety	All researchers in the Project are provided with means and equipment for personal protection at work in accordance with good laboratory practice	None	IMSI Head of Department, PI, IMSI	PIU/SF/IMSI
Project implementation	Generating plant and soil waste from inoculation with *X. campestris* pv. *campestris* and biocontrol strains in	Upon finishing the experiments, plants and soil will be disposed to autoclavable biohazard bags, heat-sterilized (autoclave) and	None	PI, IMSI	PIU/SF/IMSI

	greenhouse experiments.	discarded following strict protocols.			
Project implementation	Existence of project specific hazardous and non-hazardous chemical waste	Disposal according to waste management plan, contracted registered services for chemical waste removal, along with adoption and implementation of appropriate protocols.	None	PI, leaders of specific WPs	PIU/SF/IMSI
Project implementation	Existence of project specific microbiological waste	Contaminated waste material will be disposed to autoclavable biohazard bags, heat-sterilized (autoclave) and discarded following strict protocols.	None	PI, leaders of specific WPs	PIU/SF/IMSI
Project implementation	Proper handling of chemicals	Chemicals and other hazardous substances are managed following the guidelines provided in the safety data sheets.	None	PI, leaders of specific WPs	PIU/SF/IMSI
Project implementation	Managing equipment failure	A documented protocol outlines steps to take in case of equipment failure, overloading, or mishandling, with comprehensive records maintained accordingly.	IMSI or project overhead	PI, leaders of specific WPs	PIU/SF/IMSI
Project implementation	Fire protection	Fire-fighting equipment in place, and Instructions for evacuation routes provided according to IMSI Safety Code of Conduct and Practice Applied to Laboratories	None	PI, leaders of specific WPs	PIU/SF/IMSI

MONITORING PLAN

Phase	What parameter is to be monitored?	Where is the parameter to be monitored?	How is the parameter to be monitored/ type of monitoring equipment?	When is the parameter to be monitored frequency of measurement or continuous?	Monitoring Cost	Responsibility	Supervision
Project preparation							
Project preparation	Land use agreement required to access sampling locations.	Institution implementing the project.	By confirming the agreement has been signed before going to the field.	Once, before upcoming planned fieldwork	None	Designated person at IMSI should conduct monitoring and notify the PI.	PIU/SF/IMSI
Project preparation	Equipment for personal protection during field work (adequate clothing, gloves, safety glasses, hats, etc.).	Visual assessment in the IMSI prior to planing field activities.	Visual check if the equipment for personal protection is acquired for each team member.	Before planning field trip.	None	PI, IMSI	PIU/SF/IMSI
Project preparation	Equipment for health safety during field work – first aid kit.	Visual assessment in the IMSI prior to planing field activities.	Visual check if the equipment for personal protection is acquired for each team member.	Before planning field trip.	None	PI, IMSI	PIU/SF/IMSI
Project	Agreements	Institution	By confirming the	Once, before	None	Designated	PIU/SF/IMSI

preparation	on scientific and technical cooperation with IFVCNS for greenhouse experiments.	implementing the project.	agreement has been signed before starting greenhouse experiments.	upcoming planned greenhouse experiments		person at IMSI should conduct monitoring and notify the PI.	
Project preparation	Team members involved in field and lab work finished training activities	IMSI archives	Visual assessments and checks of dated and signed documentation in the laboratory or on-site	Once a year	None	PI	PIU/SF/IMSI
Project implementation							
Project implementation	Equipment for personal protection during field work (adequate clothing, gloves, safety glasses, hats, etc.).	Visual assessment before setting off from IMSI to field and in the field before beginning field activities.	Visual check if the equipment for personal protection is used during every field trip.	Before every field trip.	None	PI, IMSI	PIU/SF/IMSI
Project implementation	Vehicle papers are in order and valid driver licenses	IMSI	Checking documentation	Before every field trip	None	Driver	PIU/SF/IMSI
Project implementation	Equipment for health safety during field work –	IMSI	Visual check if the equipment for personal protection is used during every field trip.	Before every field trip	None	Driver, PI	PIU/SF/IMSI

	first aid kit.						
Project implementation	Deposing of plant and soil waste from greenhouse experiment	Greenhouse, IFVCNS	Check if waste management procedure are followed.	Continuously	None	PI, person in charge for waste managements in the IFVCNS	PIU/SF
Project implementation	Deposing of hazardous and non-hazardous chemical waste	IMSI	Check if waste management procedure are followed.	Continuously	None	PI, IMSI, person in charge for waste managements in the IMSI	PIU/SF/IMSI
Project implementation	Deposing of microbiologi cal waste	IMSI	Check if waste management procedure are followed.	Continuously	None	PI, IMSI, person in charge for waste managements in the IMSI	PIU/SF/IMSI
Project implementation	Emergency Response	IMSI laboratory	Review and practice the emergency response protocols, ensuring that all team members are aware of healthcare facilities and contacts	Quarterly emergency response drills and quarterly protocol reviews	None	PI, IMSI	PIU/SF/IMSI
Project implementation	Life and fire safety (LFS) procedures in laboratory	Laboratory of the institution implementing the project	Visual inspections and checks of the documentation	Periodically during the implementation of the project	None	PI, IMSI	PIU/SF/IMSI