PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XXXIX cycle, 2023-26)

Project draft

1.Field of interest

AGR/12 – Patologia Vegetale; AGR/13 – Chimica Agraria

2.Project title

Low impact technologies for plant pathogen control

3.Tutor (membro del Collegio dei Docenti)

Prof. Paola Casati

- Eventually: co-tutor/s

Prof. Barbara Scaglia

4.Relevance of the topic and state of the art:

Great increases in agricultural production and food quality have been achieved through the use of synthetic pesticides. However, they can have negative impacts on the environment and contribute to the development of pest resistance. Consequently, recent regulations limit their use and promote the use of alternative, biobased pesticides (D.L. n. 150 14/08/2022, DM 22/01/2014-PAN). These biopesticides are biodegradable and less toxic, leading to a growing interest for both pesticide and high added value vegetable production. However, the complete adoption of biopesticides is hindered by the short supply of products to meet farmers' demands, high cost of refined products, and slow action that most of them exhibit (Fenibo et al., 2021).

To make agricultural processes more sustainable, the application of circular economy (use of residues, zero waste) and green chemistry principles are fundamental. On the topic of agricultural residues and wastes, several crops (such as tomato, leek, cabbages, to name a few) produce huge amounts of wastes for which safe and economically beneficial disposal must be developed. Currently, these wastes are not properly valorized but they are known to be rich in biologically active substances that could be employed in other fields, including agriculture, if proper strategies are developed and put into practice to extract the useful compounds from these starting materials. As these molecules, for example tomatines and glucosinolates, can have an antifungal and insecticidal effect (Eugui et al., 2022; Hogland 2009; Martin-Hernandez et al., 2000) their use as potential sources of biopesticides obtained in a circular economy approach is very interesting. Despite their potential, many actions are still needed to both i) optimize their extraction to obtain an economically and environmentally sustainable product and ii) verify their effect against target pathogens and non-target organisms, to validate their efficacy as potential biopesticides.

5.Layout of the project (draft)

5.1. Materials & Methods:

The project will consist of several actions aimed at achieving the goals described above, obtaining results both concerning the production of the molecules of interest and testing in-depth their effect on different pathogens and crops, clearing their often not-completely-understood mechanism of action. To achieve this the project will include some chemistry approaches to maximize the production of extracts rich in the target active substances with the use of no organic solvents to maintain sustainability of the process.

The molecules obtained with these methods will be employed in several in vitro, in vivo, and in planta tests using pathogens of different kinds (bacteria, fungi) to check for a direct biocidal effect. Alongside these tests, the molecules will also be tested for phytotoxic effects or, on the contrary, elicitation of plant defense responses. The plant defense induction will be monitored both through the gene expression of key defense genes (e.g., NPR1) in healthy plants, and by challenging the plants with a viral infection.

The different effects obtained against different pathogens and on different crops will be investigated using molecular and biotechnological tools and – for the direct effects on microorganisms – microscopy techniques, to dissect the precise molecular mechanisms underlying the effects obtained, to not only characterize these substances as biopesticides, but also increase the general knowledge on how these plant defense compounds work.

5.2. Schedule and major steps (3 years):

At the end of each year, the following results should be achieved by the PhD student:

1st year: obtainment of plant extracts rich in bioactive compounds using methods already reported in literature; in vitro characterization of extracts against chosen target pathogens (bacteria and fungi).

2nd year: obtainment of plant extracts rich in bioactive compounds using improved methods developed *ad hoc*; in vivo characterization of extracts against chosen target pathogens and in experimental hosts; in planta characterization of phytotoxic or plant beneficial effects of plant extracts.

3rd year: dissection of molecular mechanisms underlying the effects previously described; economic and environmental analysis of the whole biopesticide production and use cycle.

6. Available funds: (*source and amount*)

The activities planned in this PhD project are in line with those of two projects that will guarantee funds necessary to allow carrying out this research. The two projects in question, SOMMELIER and BECOME are funded by Regione Lombardia and Fondazione CARIPLO, respectively, for a total of available funds amounting to approximately 350000 Euros.

7. Literature:

Eugui D., Escobar C., Velasco P., Poveda J. (2022) Glucosinolates as an effective tool in plant-parasitic nematodes control: exploiting natural plant defenses. App. Soil Ecol. 176:104497. doi: 10.1016/j.apsoil.2022.104497

Fenibo E.O., Ijoma G.N., Matambo T. (2021). Biopesticides in Sustainable Agriculture: A Critical Sustainable Development Driver Governed by Green Chemistry Principles. Front. Sustain. Food Syst. 5:619058. doi: 10.3389/fsufs.2021.619058

Hogland R. E. (2009) Toxicity of tomatine and tomatidine on weeds, crops and phytopathogenetic fungi. Allelopathy Journal, 23 (2), 425-436.

Martin-Hernandez A.M., Dufresne M., Hugouvieux V., Melton R., Osbourn A. (2000). Effects of Targeted Replacement of the Tomatinase Gene on the Interaction of Septoria lycopersici with Tomato Plants. MPMI, Vol. 13, No. 12, 2000, pp. 1301–1311