**PROJECT TITLE:** Safe Biopesticides for Protection of Minnesota Groundwater Resources

1. **PROJECT STATEMENT**

This project aims to protect ground and surface waters from dangerous pesticides by developing safer, more targeted bio-based treatments for soybean diseases. Soybean is a major crop planted on 7 million acres of land across Minnesota. Plant parasitic nematodes such as the soybean cyst nematode (*Heterodera glycines*; SCN) contribute to the largest yield losses (>30% in some fields) among all pathogens of soybean, yet there are limited methods available for their control. For many years, nematicides were used to effectively control the SCN, but many of these chemicals were recently banned due to evidence of toxic effects in humans and wildlife. For example, the fumigant dibromochloropropane is a carcinogen that can persist for nearly a decade in groundwater, while the carbamate nematicide aldicarb causes significant risks to both humans and wildlife through consumption of contaminated water and food. Other approaches to managing SCN include crop rotation, which requires longer rotations that may not be economically sustainable, and the use of resistant soybean varieties that rely heavily on a single source of plant resistance. Recent research shows the nematode is rapidly overcoming this resistance and few additional sources of plant resistance have been identified or developed, especially for northern maturity regions in Minnesota where SCN is a recent arrival. Breeding new resistant varieties can take decades and in the immediate future, farmers may be left with few alternatives but to return to use of highly toxic nematicides as there are currently no safe and effective alternatives. In order to balance the needs of farmers and the health of Minnesota groundwater, there is great need for development of bio-based and highly targeted natural chemical controls that will effectively manage these agricultural pathogens without threatening the safety and health of water resources. The Bushley and Chen laboratories have isolated over 1,000 fungi from soybean fields in Minnesota and different life stages of the nematode. Fungi isolated from nematodes are likely to be nematode parasites, many of which produce compounds that show specificity to the SCN, thus avoiding non-target effects and toxicity towards wildlife and humans. This project will use these naturally occurring fungi isolated from fields in Minnesota for development of novel bio-nematicides against this major pest of soybean to prevent contamination of Minnesota’s water resources.

**II. PROJECT ACTIVITIES AND OUTCOMES**

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| **Activity 1**: Identify compounds that inhibit nematodes from fungal isolates  **Description:** This activity will conduct chemical analysis on fungal cultures (~40) that have been previously screened and show bioactivity against the SCN by growing the fungus in a liquid broth medium, collecting the liquid filtrate, and measuring survival of SCN eggs or juvenile worms in bioassays. Fungi will be grown in two different media to produce secondary metabolite compounds. Crude extracts will be made from liquid culture filtrate and fungal tissue using both aqueous and solvent based extraction methods and the raw extract will be divided into fractions to separate out compounds. Fungal filtrates, extracts, and extract fractions will be tested for bioactivity using established laboratory bioassays to identify the active components. Analytical chemistry approaches, including HPLC, LC-MS, and nuclear magnetic resonance spectroscopy will be used to identify and structurally characterize the active chemical compounds responsible for bioactivity. Active compounds will also be tested against non-pathogenic nematodes (*Caenorhabditis elegans*) and mammalian cells to determine their target specificity and potential safety. This approach will isolate pure chemical compounds with high antagonism to the SCN that can be developed for deployment as safe and targeted nematicides. This information can also be used to develop some fungal strains as biocontrol agents in field settings.  **ENRTF BUDGET: $115,000** |  |

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| **Outcome** | **Completion Date** |
| *1. Extraction of fungal filtrates and tissue into fractions:*  40 strains x 4 extracts per sample | *November 2020* |
| *2. Bioassay testing to identify active fractions*: Nematode bioassays | *February 2021* |
| *3. Isolation of pure compounds*: Chromatography and purification of compounds, combined with iterative bioassay testing against nematodes. | *July 2021* |
| *4. Specificity testing*: Testing of the most bioactive compounds (~top 10-20) against mammalian cells and non-pathogenic nematodes | *September 2021* |

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| **Activity 2**: Test isolated chemical compounds for activity against nematodes in plant bioassays.  **Description:** This activity will test whether the most active compounds and extracts isolated in Activity 1 are effective when applied to a soybean plant grown in soil. Different methods of application of the compound, including drip irrigation, soil drench, and seed coating treatment, will be used in greenhouse potted plant assays to assess efficacy under more realistic plant growth conditions. Soybean seeds will first be sterilized and then planted into a sterilized sand/soil (70%/30%) mix. One week after emergence, each plant will be inoculated with ~1000 SCN juvenile nematodes. Treatments will consist of a control and the three different application methods, which will each be tested at three concentrations (low, intermediate, and high) based on specific toxicity in nematode bioassays. Five replicate pots, each with 4 soybean plants, will be used for each treatment. Plants will be grown for 45 days with daily watering in the greenhouse. At harvest, the number of nematode cysts and eggs will be quantified as a measure of nematode control and plant health measurements such as chlorophyll content and shoot height and biomass will also be recorded to assess any impacts on plant health. Greenhouse assays will allow us to efficiently screen a larger number of compounds to identify those that are most effective in controlling the nematode in soybean plants in preparation for field trials.  **ENRTF BUDGET: $84,000** |  |

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| **Outcome** | **Completion Date** |
| *1. Optimize method of application of best inhibitory compounds/extracts*: drip irrigation, soil drench, seed treatment | *September 2021* |
| *2. Conduct greenhouse trials using all methods and top performing compounds* | *April 2022* |
| *3. Repeat greenhouse trial with best performing compounds (top 2-3)* *and additional safety testing against non-target native species in preparation for field trials* | *June 2022* |

**III. PROJECT PARTNERS AND COLLABORATORS: N/A**

**IV. LONG-TERM IMPLEMENTATION AND FUNDING:**

This project will help protect Minnesota’s water resources from toxic pesticides by the development of more environmentally responsible and sustainable pesticides for managing important agricultural pathogens such as the SCN in Minnesota. The outcomes will be identification of active compounds, development of optimal methods for their application, and testing their efficacy in plants and safety towards other non-target organisms. This work will lay the groundwork for field trials, which we envision for Phase II of the project. Both PI Bushley and Co-PI Chen have received synergistic funding from the Minnesota Soybean Research and Production Council to screen fungal isolates for bioactivity and test biocontrol agents. The team also plans to seek additional funding from the USDA-NIFA program to sustain longer-term funding for this research.