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SUSCEPTIBILITY OF *PHILAENUS SPUMARIUS* (RHYNCHOTA, APHROPHORIDAE) TO ENTOMOPATHOGENIC NEMATODES AND FUNGI

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El-Khoury Y., Bari G., Cornara D., Poliseno M., Tarasco E. - Susceptibility of *Philaenus spumarius* (Rhynchota, Aphrophoridae) to entomopathogenic nematodes and fungi.

Xylella fastidiosa, the causal agent of the devastating "olive quick decline syndrome", is a xylem-inhabiting gram-negative bacterium that was introduced into the Apulia region of southern Italy. *Xylella fastidiosa* caused an epidemic outbreak and catastrophic dieback of olive groves. This pathogen is transmitted exclusively by xylem fluid-feeding sap insects, the main vector being the meadow jumping beetle *Philaenus spumarius*. The use of microorganisms such as entomopathogenic nematodes and fungi is a well-known biocontrol strategy against agricultural pests. This study aims to evaluate the pathogenicity of two different entomopathogenic fungi (EPF), the commercial *Beauveria bassiana* strain ATCC74044 (Naturalis®) and *Lecanicillium muscarium* Ve6 (Mycotal®), and two indigenous strains of EPNs against juveniles of the meadow spittlebug *Philaenus spumarius*. Monitoring of insect vectors is necessary to prevent the further spread of *Xylella fastidiosa* in Europe. The rapid spread of *Xylella fastidiosa* underlines the importance of preventive biocontrol measures against the vector with EPNs and EPF.

Key Words: *Xylella fastidiosa*, insect vector, *Philaenus spumarius,* entomopathogenic fungi entomopathogenic nematodes

INTRODUCTION

Xylella fastidiosa (Proteobacteria: Xanthomonadaceae), the causal agent of the devastating olive quick decline syndrome is a xylem-inhabiting gram-negative bacterium (EPPO, 2016). This pathogen obstructs the xylem of the tree that shows a range of symptoms starting with leaf necrosis, scattered desiccation of twigs and branches descending from the top of the tree canopy to the death of the trees within a few years from the onset of symptoms (Schneider *et al.*, 2020; Bucci, 2018). It was reported for the first time in Europe in 2013 in the Apulia region of southern Italy. Later it was detected in several countries in the EU, including Italy, France, Portugal, and Spain (EFSA, 2018). This pathogen is transmitted exclusively by xylem fluid-feeding sap insects, the main vector being the meadow spittlebug *Philaenus spumarius* L. (Hemiptera: Aphrophoridae) that is threatening several perennial crops, including vineyards and olive and almond groves (Avosani *et al.*, 2023). The control of the epidemiology of this vector-transmitted plant disease, depends closely on controlling the spread of the vectors as there is no treatment against *X. fastidiosa*. The use of microorganisms such as entomopathogenic nematodes and fungi is a well-known biocontrol strategy against agricultural pests but very few studies tested their pathogenicity against *P. spumarius* (Kanga *et al*., 2004; Vicente-Díez *et al*., 2021). This study aims to evaluate the pathogenicity of two different entomopathogenic fungi (EPF), the commercial *Beauveria bassiana* strain ATCC74044 (Naturalis®) and *Lecanicillium muscarium* Ve6 (Mycotal®), and two indigenous strains of EPNs against juveniles of the meadow spittlebug *Philaenus spumarius*. Monitoring of insect vectors is necessary to prevent the further spread of *X. fastidiosa* in Europe. The rapid spread of *X. fastidiosa* after its introduction underlines the importance of preventive biocontrol measures against the vector with EPNs and EPF.

MATERIAL AND METHODS

The experiments were performed with collected nymphs from the field and adults reared in the laboratory. Plants with spittle on that indicate the presence of the nymphs were collected, particularly *Foeniculum* sp. and *Glebionis* sp. Adults were procured from a colony reared on basil plants in the CIHEAM Bari institute.

Two preliminary bioassays were carried out to test the efficacy of different biological entomopathogenic agents against nymphs and adults of the meadow spittlebug *Philaenus spumarius* using the entomopathogenic fungi *Beauveria bassiana* strains ATCC74044 (Naturalis®) and *Lecanicillium muscarium* Ve6 (Mycotal®) and two indigenous strains of EPNs *Heterorhabditis bacteriophora* LP12 and *Steinernema feltiae* BAR8. The first was carried out *in vitro* against immature stages of *P. spumarius* using 8 insects per treatment, 100 IJs/nymph EPN or 4.10^4 spores/mL EPF (both water suspensions); the mortality was checked after, 2, 3, and 4 days. The second was carried out on *P. spumarius* adults incubated

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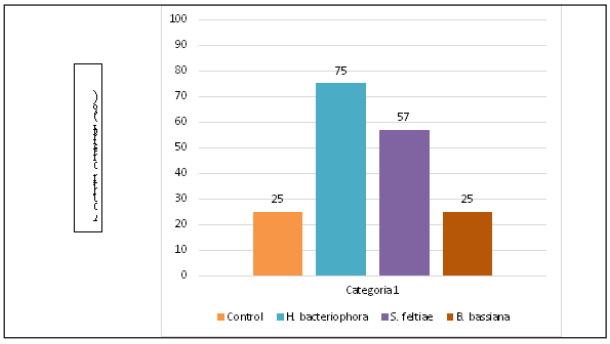

Fig. I - Mean mortality of *Phileanus spumarius* adults on treated vetch plants

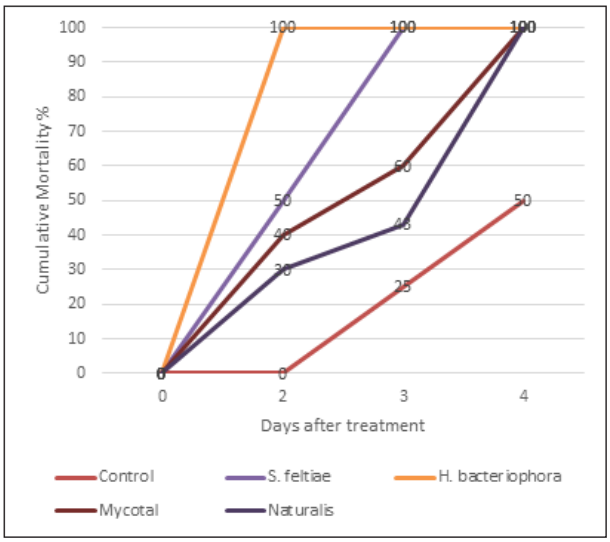

Fig. II - Mean mortality of *Phileanus spumarius* nymphs after exposure to different treatments in Petri dishes

on vetch plants treated with EPF and EPN. The mortality was recorded after 5 days.

RESULTS AND DISCUSSION

All treatments caused the total mortality of the nymphs after 4 days, however, only EPNs were found inside the insect's cadaver (Fig. I). The highest and quickest mortality was induced by *H. bacteriophora,* and lower adult mortality rates were recorded on treated plants (Fig. II). The virulence of EPN was considerably higher than the virulence of EPF against the adults of *P. spumarius.* Our results could lead to the conclusion that the spittle can act as a facilitator for nematodes to penetrate their host, but protect the nymphs against the EPF spores.

Monitoring of insect vectors is necessary to prevent the further spread of *X. fastidiosa* in Europe. The rapid spread of *X. fastidiosa* once introduced, underlines the importance of preventive biocontrol measures against the vector with EPNs and EPF.

The presence of *P. spumarius*, which plays the main role in the transmission of *X. fastidiosa* subspecies *pauca* in the Apulia region of Italy, ensures the dissemination of the bacterium when it is present; therefore, additional monitoring and control measures of propagation materials and continuous evaluation of potential vectors of *X. fastidiosa* are needed to prevent its introduction and dissemination to other regions and countries.

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