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Volatile Compounds and Enzymatic Activity in the Biocontrol of Yellow Wilt in *Piper nigrum* by *Bacillus velezensis* PN2-45

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Black pepper (*Piper nigrum*), an important spice of high commercial value with Brazil and Pará state as one of the world's leading producers. However, its cultivation is severely affected by yellow wilt caused by *Fusarium oxyspo*rum. In this sense, this study evaluates the potential of *Bacillus velezensis* PN2-45 to control *F. oxysporum* infection in *P. nigrum* plants. Previously, the strain *B. velezensis* PN2-45 inhibited 72.9% of the mycelial growth of *F. oxysporum* in vitro by dual culture assay and the GC-MS analysis identified its volatile compounds reported as antifungal agents, including dodecanal, n-decanal, 4-(2,5,6,6-tetramethyl-1-cyclohexen-1-yl)-3-buten-2-one, and 2-ethyl-hexanol. *Piper nigrum* cv. Bragantina plants were coinoculated with *B. velezensis* PN2-45 and *F. oxysporum* under greenhouse, and the samples were collected at 3 and 9 weeks after inoculation. Co-inoculation reduced yellow wilt symptoms in *P. nigrum* seedlings, while infected plants exhibited leaf yellowing and premature defoliation. Leaf volatiles included α-muurolol, γ-cadinene, bicyclogermacrene, and *epi*-α-muurolol, whereas root volatiles comprised β-caryophyllene, δ-elemene, camphene, and α-muurolol. The co-inoculation altered the seedlings' volatile chemical profile: the levels of β-caryophyllene were significantly impacted by fungal infection, showing an increase after 3 weeks (from 16.96% to 29.33%) and after 9 weeks (from 19.51% to 33.16%) compared to control. In contrast, co-inoculation led to a significant reduction in β-caryophyllene levels in root samples compared to pathogen-infected seedlings. After 3 weeks, the levels decreased from 29.33% to 21.89%, and after 9 weeks, they reduced from 33.16% to 26.29%. High amount of β-caryophyllene is associated with increased susceptibility to fungal infection, also low levels of this sesquiterpene in vitro (100 $ng.\mu L^{-1}$) promote the faster hyphal growth of *F. graminearum,* suggesting that some pathogens can modulate the host's synthesis of this compound for their own benefit[1]. These results suggest that the inhibition of this compound's synthesis by the PN2-45 strain may aid in the biocontrol of yellow wilt. Inoculation with *B. velezensis* PN2-45 stimulated the activity of phenylalanine ammonia-lyase (44.8-60.9 $U.mL^{-1}$) and the lipoxygenase (4.2-6.5 $M.s^{-1}$) pathway in leaves after 9 weeks and highly increased the total phenolic content in roots samples, ranging from 3.8 $mgEAG.g^{-1}$ to 14.79 $mgEAG.g^{-1}$ after 3 weeks. These results suggest a potential role in resistance induction in *P. nigrum*. In conclusion, the *B. velezensis* PN2-45 strain shows great potential for agronomic applications in the biocontrol of yellow wilt.

Keywords: *black pepper, biological control, Fusarium oxysporum, antibiosis*

[1] *Fantaye, C.A., et al.* ***Journal of Chemical Ecology****, v. 41, p. 213-223. 2015.*