ABSTRACT

The objective of this study was to select and test the efficacy of *Bacillus* spp. antagonist in inhibiting the growth of *Alternaria* spp., the causal agent of lettuce leaf spot disease in hydroponic farms and to prepare the fresh cells of *Bacillus* spp. into a water-soluble granule for spray application to suppress leaf spot disease. Fifteen isolates of *Alternaria* spp. were isolated from leaf spot disease samples of lettuce obtained from 14 hydroponic farms in several provinces of Thailand using tissue transplanting method. *Alternaria longipes* (isolate LRC 4–6) was the most virulent strain to the lettuce plants, comparing to other isolates when pathogenicity test was undertaken. A total of 356 isolates of *Bacillus* spp. were isolated from forest soil samples in Thailand using soil dilution plate method. These *Bacillus* spp. isolates were screened to detect their effectiveness in inhibiting mycelial growth of *A. longipes* LRC 4–6 using dual culture technique on PDA medium. Eighteen isolates of *Bacillus* spp. showed the potential in antagonizing *A. longipes* LRC 4–6, based upon their effect on inhibiting a mycelial growth and spore germination. *Bacillus subtilis* isolate LPDD 3–1, was the most effective in inhibiting mycelial growth (with 97.6% and 95.6% reduction) when sterilized and non-sterilized supernatant was incorporated into the PDA double strength medium respectively. This bacterium also was the most effective in inhibiting spore germination (with 91.6% and 86.3% reduction) when spores were suspended in water mixed with either sterilized or non-sterilized supernatant respectively. Moreover, the conidia treated with these supernatants had obvious abnormal morphology with shortened germ tubes and cell swelling.

Subsequently, five water-soluble granule formulations were successfully prepared using wet granulation method. The suitable and applicable formulation composed of sodium alginate (12.5%), PVP (k–30) (12.5%), lactose monohydrate (75%) and B.
*B. subtilis* LPDD 3-1 endospore suspension (at $4.4 \times 10^{15}$ cfu). This formulation exhibited good physical characteristics with high solubility, high viscosity (at 18.90 cps) and neutral pH. Sprayed upon a lettuce leaf, the bacterial population still remained high when a number of the bacterium was assessed 10 days after application. After being stored for 6 months, a number of the bacterium in the formulation was at $10^9$ cfu/g (at 26–30°C). Under laboratory condition, an aqueous solution of the formulation showed high activity in inhibiting mycelial growth of *A. longipes* LRC 4-6 (with more than 95% reduction).

The testing to determine the efficacy of the water-soluble granule formulation under hydroponic greenhouse condition was arranged in a completely randomized design (with 9 treatments and 5 replications). It was found that spraying the formulation 1 day before pathogen inoculation, followed by spraying the formulation again at 3 and 5 day after pathogen inoculation, had high potential for deterring and suppressing the development of lettuce leaf spot disease. After spraying the lettuces with the developed formulation, % leaf with disease symptom/plant in green oak, red coral and butter head was at 20.0%, 19.4% and 22.7% respectively, with the reduction in % leaf with disease symptom/plant at 53.2% (for green oak), 57.9% (for red coral) and 65.9% (for butter head) when comparing with the non-treated control. The effect of the spray also increased the percentage of fresh weight of the lettuces at 57.1% (for green oak), 43.4% (for red coral) and 49.7% (for butter head).