Effect of spray application parameters on viability of rhizobacteria used as bio-pesticides in organic fruit production

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Introduction

The biocides, either synthetic pesticides or biopesticides, are applied with agricultural sprayers in form of an atomised spray liquid, being usually a solution or suspension of the control agent, i.e.: synthetic active ingredient, microorganism or extract of naturally occurring material. Prior to the atomisation the spray liquid is subject to a turbulent flow and series of processes taking place in different sprayer components. In the pump the liquid is in turns compressed and decompressed at the frequency of 10-50 Hz (diaphragm or piston pumps) or subject to mechanical rotation at high speeds of 1500-3500 RPM (centrifugal pumps). In the filters the compressed liquid passes the mesh, in the control devices such as valves and gauges it flows at high speed passing sharp edges, and finally in the nozzles it is atomised by mechanical breaking or tearing apart. During all these processes the sprayer components and their working parameters harshly interact with the particles suspended in the liquid and usually they cause an increase of the liquid temperature. The leaving micro-organisms in biopesticides are subject to unfavourable conditions being far from those of their natural habitat. Thus, the question about viability of microbes under these conditions is very important for the efficacy of biopesticides. The objective of this study was to evaluate the survival of two strains of PGPR (Plant Growth Promoting Rhizobacteria), showing potential of application in organic fruit growing, by performing the stress tests simulating spray application process with both the hydraulic and the pneumatic atomisation system.

Material and Methods

Two strains of the Gram-negative bacteria, *Pseudomonas fluorescens* (PS49A) and *Enterobacter nimipressuralis* (K50XA), were isolated from the rhizosphere of two species of the genus *Fragaria*, respectively: *F. ananasa* (garden strawberry) and *F. vesca* (wild strawberry). For the bacterial viability evaluation tests appropriate volumes of spray liquid were prepared, being a bacterial suspension with the concentration of bacteria $40 \div 100 \times 10^4$ CFU/ml (colony forming units per millilitre of suspension). A test stand was designed and developed to control and measure the parameters of flow of bacterial suspension during the simulated spray application (Fig. 1). The stand included two independent application systems used in fruit crop sprayers: (i) hydraulic atomisation system with a high pressure diaphragm pump and 16 hydraulic hollow-cone nozzles TR80 (Lechler) - in the experiment tested at the pressures 0.5, 1.0 and 1.5 MPa and at the spray volume rate 450 $l \cdot ha^{-1}$ (in the additional tests also at spray volume 55 $l \cdot ha^{-1}$); (ii) pneumatic atomisation system with a low pressure centrifugal pump, a radial fan and four Paraflow pneumatic atomisers (Hardi) - tested at the pressures 0.1 and 0.25 MPa and at spray volume rates 55 and 125 $l \cdot ha^{-1}$. Each system consisted of adequate pressure control devices and components assembled and linked as in commercial sprayers in order to best simulate spray application in real situations. In both systems the critical parameters of the liquid flow and atomisation, such as flow intensity, liquid pressure, and air jet velocity were all controllable. The pressure, the temperature and the flow of liquid in different sites of the liquid circuits were monitored and registered by a software application. While the application systems were operating the samples of the bacterial suspension were taken at different sampling sites, at 15 minute intervals. The samples were serially diluted (10^{-1}, 10^{-2},..., 10^{-4}), and the diluted samples were spread (0.1 ml each) on petri dishes, containing KingB agar medium. After 72 hours of incubation at a temperature of 28°C the bacteria colonies growing on the agar medium were counted. The population of bacteria was expressed as the colony forming units (CFU).

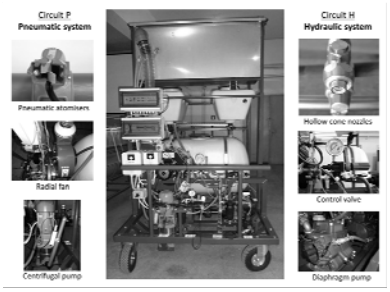

Fig. 1 Test stand with two independent liquid circuits to simulate application parameters during the stress tests on viability of rhizobacteria in biopesticides: Circuit P with the pneumatic atomization system; Circuit H with the hydraulic atomization system

Results

The viability of bacteria applied at normal application conditions imposed by both atomisation systems was in most cases above 90%, and with only a single low viability of 82%. In such conditions the temperature of the bacterial suspension was only slightly increased during the sprayer operation and therefore it was not considered a crucial factor. Neither application pressure nor the components of the liquid circuits and atomisation systems of the test stand showed alone any decisive effect on the viability. The bacterial mortality was mainly due to the interaction of the pressure, time, and the components. In the extremely harsh conditions of very intensive circulation of bacterial suspension in the liquid circuit of the hydraulic application system (additional test: the worst case condition; spray volume rate 55 $l \cdot ha^{-1}$) the temperature of the bacterial suspension increased dramatically, thus in combination with a high pressure caused 50% bacterial mortality after 70 minutes, and total mortality after 100 minutes.

Conclusions

The two evaluated strains of bacteria *Pseudomonas fluorescens* (PS49A) and *Enterobacter nimipressuralis* (K50XA) can be successfully applied by sprayers equipped with either hydraulic or pneumatic atomisation system, which are commonly used in fruit growing. The viability of bacteria applied in form of bacterial suspension was satisfactory when applied at normal application conditions. Only situations imposing very harsh conditions, e.g. long and intensive liquid circulation in the sprayer liquid system should be avoided in order to maintain satisfactory viability of the two evaluated bacteria strains.