Influence of Mechanical Aspects of Distribution on Viability of the Biological Control Agent *Steinernema carpocapsae*

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Abstract
The distribution of entomopathogenic nematodes (EPNs) for pest control in organic and conventional agriculture is usually carried out with traditional boom sprayers normally used for the distribution of pesticides. Nevertheless there is a reasonable possibility that this operation may induce a state of stress due to sudden pressure changes and mechanical stress, especially during the passage through the pump and nozzles or as a consequence of the effect of hydraulic or pneumatic agitation inside the tank.
This work aims to evaluate the effects of some mechanical distribution parameters on the viability of the entomopathogenic nematode *Steinernema carpocapsae* Weiser which may become significant during the distribution.
To assign the mechanical effects to specific components of the distribution, liquid suspensions of the nematode were subjected to different levels of static pressure, and various conditions of agitation and distribution using different types of nozzles.
Preliminary results show no significant effect of static pressure on the viability of nematodes. The passage through the nozzles and the use of a elastic rotor pump induces a 7% decrease in viability, but no difference was detected between different nozzles, while the intensity of hydraulic agitation appeared to affect the nematodes viability.

Keywords: Application technique, Entomopathogenic nematodes, Organic agriculture, Pest control

Introduction

The entomopathogenic nematode *Steinernema carpocapsae* plays an important role in the biological and integrated control of various phytophages (in particular the larvae of lepidopterans that attack fruit trees, noctuids, coleopterans and orthopterans). In Italy it is currently successfully used in the biological control of two new parasites on palms: the Asian palm weevil, *Rhynchophorus ferrugineus* Olivier, and *Paysandisia archon* Burmeister. The nematode can be distributed with the irrigation or by means of mechanical distribution on the crop or directly on the soil.

Normal sprayers are commonly used for the mechanical distribution of these antagonists, but there is a real possibility that this operation may induce a state of stress due to abrupt pressure variations in the tank and mechanical stress, which the organisms are subjected to in different points of their route inside the machine, particularly while passing through the pump and nozzles. Further mechanical stress may be caused by the effect of the hydraulic or pneumatic agitation inside the tank (Nilsson and Gripwall, 1999; Łaczyński et al., 2004) and by the rise in temperature produced by the agitation system mixing the suspension (Łaczyński et al., 2006). However, studies on the reduction in viability of the nematodes following mechanical distribution do not provide uniform results. Whereas Fife et al. (2003, 2004) reported that a single passage through different types of pump, when the pressure difference is no greater than 13.8 bars, did not influence the viability of *Heterorhabditis bacteriophora*, *H. megidis* and *Steinernema carpocapsae*, Nilsson and Gripwall (1999) observed a 10% reduction in viability of *S. feltiae* after passing through a piston pump. Grewal (2002) reported the negative effect of excessive hydraulic agitation on the viability of nematodes and Łaczyński et al. (2004) indicated a linear decrease in the viability of *H. bacteriophora* with respect to the duration of the hydraulic agitation.

This paper evaluates the effects of some mechanical parameters of the distribution on the viability of entomophages when undergoing different levels of mechanical stress that may occur during distribution. To attribute the mechanical stress caused by the specific components of the distribution (static pressure, dynamic pressure, mixing), 3 different trials were conducted to evaluate the influence of the following mechanical aspects on nematode viability:

- static pressure;
- response to the distribution using a traditional sprayer with different types of nozzles;
- effect of the pump and agitator.

Materials and methods

A commercial formula, NemoPAK-S®, of desiccated *S. carpocapsae* larvae was used, containing 625,000 individuals per gram in a mixture of chitosan (a polysaccharide obtained from the chitin of crustaceans). Prior to each trial the nematodes were rehydrated by diluting 2 g of product in 1 litre of water (22 °C) from an artesian well to simulate the usual composition of the suspension distributed on the field. A sample of 0.25 litres was then further diluted in 5 litres of water to obtain a final concentration in the suspension of 62,500 individuals per litre.

For each test, a sample of 100 ml of suspension was taken and stored in plastic test tubes in the dark at a temperature of 14-16 °C for 18-20 hours to limit the reduction in nematode survival following conservation (Molyneux, 1985). Around 30 minutes before the count, a sample of nematodes (1 ml) was extracted using a calibrated pipette and left at ambient temperature in the dark to encourage mobility and facilitate the counting of the individuals (Łaczyński et al., 2006). The sub-samples, diluted with 3 ml of distilled water, were placed in Petri dishes with a grid base, and the nematodes were counted with the aid of a binocular microscope (model Zeiss 12x - 100x), lighting the sample with an incandescent lamp that projected the light from top downwards to avoid causing the death of any not very lively individuals. Only whole nematodes were counted; fragments were not considered as they may have already been present in the commercial formula (Łaczyński et al., 2006). The nematodes were identified as alive based on their active movement (Grewal, 2002) and a needle was used to stimulate those that appeared immobile. For each sample the relative viability, V_r, was determined as a percentage of the total number of nematodes.

2.1 Evaluation of the effect of static pressure

The effect of static pressure was reproduced by means of a test bench composed of a 150 ml capacity metal container connected to a manually operated hydraulic jack, equipped with a manometer. The instrument allowed the pressure applied to the suspension to be graded and the load maintained for the specified times (figure 1).

Figure 1. Equipment for the application of static pressure

During this trial viability of the nematode was evaluated subjected to levels of static pressure comparable to those which the organism is subjected to during traditional distribution. Six treatments were considered characterized by four pressure levels (0, 2, 8 and 14 bar) and different times of application of the intermediate pressure (table 1). Each treatment was replicated three times.

Table 1. Resistance to static pressure, pressures and times applied

treatment	pressure (bar)	time (s)
1 test	0	0
2	2	15
3	14	15
4	8	5
5	8	15
6	8	25
7	8	35

When the pressure had been applied for the established time, a sample of suspension was taken from a valve situated in the lower part of the compression chamber. The relative viability in the samples was then measured according to the trial protocol.

2.2 Evaluation of mechanical stress caused by passing through the nozzles

A test bench was set up to examine, in the laboratory, the effect of the nematode suspension passing through different types of nozzle.

Three types of TeeJet XR flat fan nozzles (TeeJet, Spraying Systems, Wheaton, ILL, USA) of different sizes were considered (table 2). To avoid the introduction of any mechanical stress in addition to that caused by passing through the nozzles, a flexible impeller pump (Liverani mod. INV MIDEX 3/4) was used without a filter, which guarantees, thanks to its construction characteristics, the absence of peaks of pressure that occur next to the valves in diaphragm and piston pumps. The electric motor of pump was equipped with a frequency converter for a continuous regulation of the rotational speed (range 180 and 1400 revs/min). Intake was through a pipe sucking the suspension from a mixing tank in which the product was maintained in constant agitation. The pipe was connected to a boom fitted with only one nozzle, which was changed for the different treatments.

Table 2. Test conditions of passage through the nozzles

treatment	nozzle	colour code	flow (l/min)	pressure (bars)	revs/min
1 test	-	-	-		-
2	XR11008	white	3.16	3	1400
3	XR11004	red	1.58	3	1150
4	XR11001	orange	0.39	3	900
5	-	-	88	0	1400

A control was also considered, taken directly from the central part of the tank. In the fifth treatment the sample was taken without the passage through the nozzle, in order to point out any difference in viability between the effect of the double passage through pump and nozzle, and the single passage only through the pump. All treatments were replicated three times.

2.3 Evaluation of the mechanical stress caused by the recirculation

A test bench was set up in the laboratory to check the combined effect of the recirculation in the tank and the delivery through the nozzles on the viability of the nematodes. The trial aimed to demonstrate any contribution of the ejector to the death of nematodes following the mixing of the suspension.

The system was composed by a 300-litre capacity tank equipped with a hydro-ejector hydraulic mixer, a traditional boom sprayer, with constant pressure regulation system, equipped with flat fan nozzles and a low-pressure piston-diaphragm pump (Comet BP 75). The filter installed between the pump and the tank was removed to allow the nematodes to pass through.

Figure 2. Boom sprayer used for the trials of resistance to agitation in the tank

To increase the mixing action of the hydraulic ejector, which was insufficient at the low pressures used, the return circuit in the tank was shut with a valve. Two agitation levels were considered, L_1 and L_2, obtained with pump speeds of 252 and 380 revs/min respectively. The flow-rates and the pressures of this trial are reported in table 3.

Table 3. Test conditions of agitation in the tank with a traditional sprayer

level of agitation	ejector flow-rate (l/min)	pump speed (revs/min)	delivery pressure (bar)
L_1	23.9	252	3±0.1
L_2	36.9	380	3±0.1

For both agitation levels, 4 treatments were considered (table 4). The first (control) was a sample taken directly from the tank. The other treatments were taken, at the outlet of the nozzles, at successive intervals corresponding to decreasing volumes of the suspension in the tank: 75%, 50% and almost empty tank. These levels of volume delivered correspond to increasing amount of recirculation and mixing times. The pressure was maintained at 3 ± 0.1 bar throughout the time of delivery. Each sampling was replicated three times.

Table 4. Test of resistance to agitation. It is reported the theoretical average number passages of each organism in the agitation-return circuit throughout the emptying cycle

treatment	level in the tank (l)	average number of passages	
		L_1	L_2
1 test	300	0	0
2	225	0.11	0.16
3	150	0.16	0.25
4	25	0.96	1.47

Results

<u>*3.1 Effect of the static pressure*</u>

The average values of relative viability are between 58.1% for the sample subjected to a pressure level of 8 bar for 5 seconds and 67.8% for the control (table 5).

The results did not demonstrate any variation in the viability of the organism related to mechanical stress. In particular the static pressure had no significant effect on the viability of the nematode within the range considered (2, 8 and 14 bar). Different durations of application of the pressure of 8 bars (5, 15, 25, 35 seconds) did also show no significant differences.

Table 5. Effect of static pressure on the relative viability (V_r) of the organisms

treatment	pressure (bar)	time (s)	V_r (%)	homogeneous groups
1 test	0	0	67.8	a
2	2	15	59.9	a
3	8	5	58.1	a
4	8	15	58.9	a
5	8	25	64.1	a

6	8	35	61.0	a
7	14	15	64.0	a

<u>*3.2 Mechanical stress caused by passing through the nozzles*</u>

The combined effect of the mechanical stress caused by the elastic rotor pump and by the mixture forced through the nozzles shows that the viability of the control is higher (81.3%) than and significantly different ($p<0.05$) from all the samples subjected to mechanical stress, including the case of a single passage through the pump at free discharge (75.6%).

The use of different types of nozzles did not significantly affect the viability of the nematode and did not increase the mortality with respect to the single passage through the pump (figure 3).

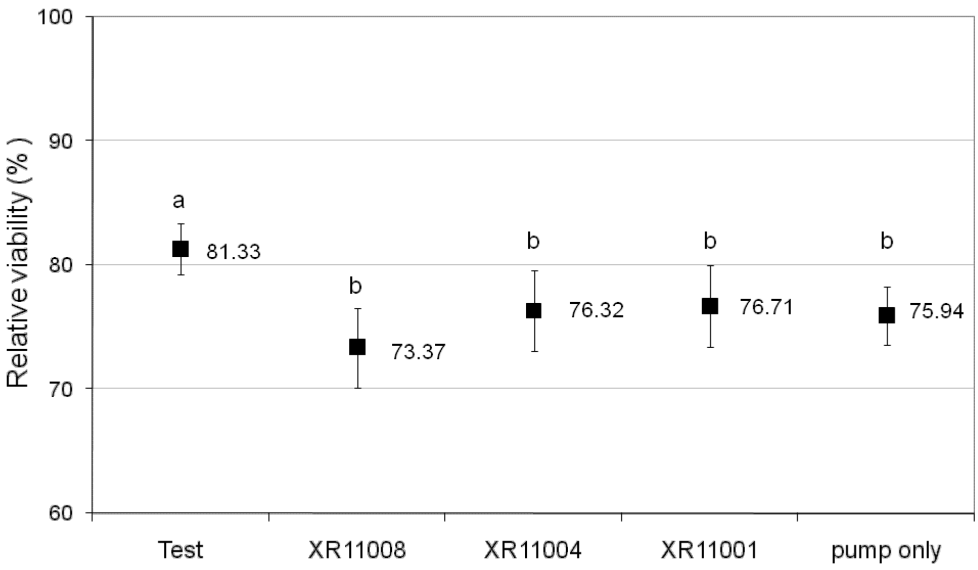

Figure 3. Viability of nematodes that passed through the nozzles (vertical bars represent the 95% confidence interval)

<u>*3.3 Mechanical stress caused by traditional distribution*</u>

Lastly, the nematodes subjected to two agitation systems did not show any reduction, but in both trials, when the tank was nearly empty, their concentration in the suspension was about 20% higher than the initial one, sign of a not quite satisfactory agitation.

Table 6. Results of the check on the viability for the two levels of agitation-return (the homogeneous groups are calculated for $p<0.05$)

test	level of the tank (l)	viability (%)	homogeneous groups	viability (%)	homogeneous groups
		agitation level L_1		agitation level L_2	
1 test	300	89.2	a	94.5	a
2	225	88.3	a	91.1	b
3	150	85.1	a	92.4	b
4	25	87.0	a	91.2	b

Conclusions

The results of the trials demonstrated that a static pressure up to 14 bar, even protracted for some seconds, causes no significant damage to the specific organism examined (*S. carpocapsae*). It was not considered worth testing any higher pressure levels as the use of flat fan nozzles was hypothesized, which normally operate at maximum pressures of 3 or 4 bar. This type of nozzle, used at low pressure, can be considered suitable for the distribution of live nematodes, due both to the relatively large size of the drops that are produced, and the absence of the core, typical of the cone-nozzles, which would make the flow of the liquid more turbulent.

The effect of passing through the nozzles was verified using a elastic rotor pump. The choice of this pump was initially suggested by the hypothesis that the other traditional pumps would produce anomalous peaks of pressure, much higher than the average pressure. In this way it was thought to be able to isolate the effect of the nozzles from that of the pump. The results appear instead to demonstrate that the slight difference in the viability is to be ascribed more to the pump, than to the calibre of the nozzles. On the other hand, the trials of recirculation carried out with the traditional sprayer have shown that repeated passages of the organisms in the hydraulic system may affect their viability more than other factors, including the diaphragm pump.

It can therefore be considered that in normal pressure conditions and with flat fan nozzles of any calibre, *S. carpocapsae* may be safely distributed with traditional sprayers, maintaining the level of the agitation-return low, perhaps reducing the rpm of the p.t.o. and taking into account an increase in concentration when the tank is almost empty.

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