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Comparative assessment of *Pasteuria penetrans* and three nematicides for the control of *Meloidogyne javanica* and their effect on yields of successive crops of tomato and melon

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Summary – The effects of methyl bromide, itaconate dimethyl (ID), cadusaphos, and *Pasteuria penetrans* on *Meloidogyne javanica* reproduction and gall index and yield of three successive vegetable crops were evaluated. Only methyl bromide treatments achieved a drastic reduction of nematode populations, resulting in low galling indices and significantly increased yield. Soil recolonization by nematodes during the second year was important and caused yield reduction. ID had no nematicidal effect but caused a slight yield increase. Cadusaphos had a delayed effect on nematode build-up and significantly increased yields. During the first crop, *Pasteuria penetrans* had no direct effect on nematode populations, gall indices, and yield, but it multiplied actively in the soil. This effectively suppressed nematode populations and reduced gall indices on successive crops, which increased yield. © Elsevier - ORSTOM

Résumé – ***Comparaison de l'action de* Pasteuria penetrans *et de trois nématicides sur le contrôle de* Meloidogyne javanica *et sur les rendements de cultures successives de tomate et de melon*** – Les effets du bromure de méthyle, du diméthyle itaconate (DI), du cadusaphos et de *Pasteuria penetrans* ont été évalués sur les taux de populations de *Meloidogyne javanica*, l'indice de galles et les rendement de trois cultures maraîchères successives. Seul le traitement au bromure de méthyle permet une forte réduction des densités de populations de nématodes, d'où résultent un faible indice de galle et une augmentation significative du rendement. La recolonisation du sol par les nématodes durant la deuxième année est plus importante, ce phénomène étant associé à une forte diminution du rendement. Le DI n'a pas d'effet nématicide mais permet une légère augmentation du rendement. Le cadusaphos a un effet retard sur les populations de nématodes et permet d'améliorer significativement le rendement. *P. penetrans* n'a d'effet direct ni sur les nématodes, ni sur l'indice de galles ou le rendement lors de la première culture. Ce traitement biologique permet une multiplication active du parasite dans le sol durant cette première culture, mais il réduit les densités de populations de nématodes et l'indice de galle des cultures suivantes, tout en améliorant le rendement. © Elsevier - ORSTOM

Keywords: cadusaphos, itaconate dimethyle, *Meloidogyne javanica*, melon, methyle bromide, parasitism, *Pasteuria penetrans*, tomato.

The root-knot nematode *Meloidogyne javanica* is one of the most important constraints for the production of vegetable crops in plastic greenhouses in Morocco (Eddaoudi *et al.*, 1997). Nematicides are expensive, some are dangerous, and their effectiveness is irregular. Resistant cultivars are available only for tomato and pepper crops, and populations of *M. javanica* have been shown to overcome the resistance genes of resistant tomato in case of monoculture (Eddaoudi *et al.*, 1997).

Pasteuria penetrans is an obligate parasite of *Meloidogyne* spp. (Starr & Sayre, 1988). It is an effective and promising biological control agent that acts by reducing nematode development; its beneficial effects on plant growth and production have been observed in the field on crop sequences and perennial crops (Brown *et al.*, 1985; Bird & Brisbane, 1988; Oostendorp *et al.*, 1991; Verdejo-Lucas, 1992). The suppressive effect of *P. penetrans* depends on the density of spores in the soil. *P. penetrans* acts in three ways: reduction of nematode motility, restriction of root penetration, and reduction of egg mass production (Starr & Sayre, 1988; Kasumimoto *et al.*, 1993). The bacterium has a world-wide distribution (Starr & Sayre, 1988; Sturhan, 1988). In Morocco, spores are sometimes observed on juveniles of *Meloidogyne* spp. and other nematode genera, but the rate of parasitism is generally less than 0.5% (Eddaoudi, unpubl.).

The present study was carried out to evaluate *P. penetrans* (actinomycete), methyl bromide, itaconate dimethyl (ID), a new synthesized molecule derived from a mycotoxin secreted by *Paecylomyces lilacinus*, and Rugby 10G (cadusaphos) for the control of *M. javanica* populations and their respective effect on the yield of three successive crops (tomato, melon and tomato).

Materials and methods

The study was made in a greenhouse heavily infested by *M. javanica* and previously cultivated with cucumbers. The greenhouse was divided into plots of 18 m^2 (4 × 4.5 m) separated by a 200 µm polyethylene film at 0.40 m depth in soil.

Experiments were established in sandy soil (86.7 % sand, 7.3 % clay, 6 % silt) with an organic matter content of 0.5 %, pH 8.5, and CEC of 3.7 meq/100g.

A completely randomized design with four treatments and four replications was used: *i*) *Pasteuria penetrans* isolate ORSTOM PPSN14007 originating from Senegal and capable of parasitizing *M. javanica*, *M. artiellia*, *M. arenaria*, and *M. hapla* (Bourijate & Ciancio, 1995); *ii*) methyl bromide (MB) applied at 70 g/m^2 1 month before planting; *iii*) itaconate dimethyl (ID) applied in water suspension at 3 g/m^2 on the soil surface 6 h before planting; the soil was watered after application to favour penetration of the product; *iv*) control. The first three treatments were applied only on the first crop. Cadusaphos (Rugby 10G) was applied at 4 g/m^2 on soil surface before the planting of the third crop, only in plots previously treated with ID.

Pasteuria penetrans inoculum was prepared according to Stirling and Wachtel (1980). A mixture of soil with 500 g of root powder with spores of *P. penetrans* was prepared in the laboratory to serve as carrier and substrate for the multiplication of the bacteria. Roots of young plants were coated with 500 cm^3 of this mixture at planting.

The experimentation lasted 2 years on three nematode susceptible crops: tomato cv. Eclaireur planted June 26, 1992, melon cv. Gallia planted January 11, 1993, one month after tomato harvest, and tomato cv. Daniela planted October 4, 1993, two months after melon harvest. At the end of each crop, roots were left to decompose in the soil. Before planting a new crop, all plots were superficially raked to air the soil. Each

Table 1. *Comparison of biological and chemical control: the number of* Meloidogyne javanica *juveniles per dm^3 of soil extracted during three succesive vegetable crops*

Sampling dates	*Pasteuria penetrans*	Itaconate	Cadusaphos	Methyl bromide	Control
Tomato cv. Eclaireur					
20-05-92	760 *bc A*	30 *bc A*	-	790 *a A*	820 *a A*
26-06-92	933 *c B*	752 *bc B*	-	0 *a A*	710 *a B*
23-07-92	3250 *e B*	3000 *e B*	-	0 *a A*	3800 *b B*
19-08-92	5525 *f D*	4650 *f C*	-	78 *a A*	3850 *b B*
23-09-92	1660 *d D*	2310 *d B*	-	102 *a A*	5800 *bc C*
09-11-92	64 *a A*	217 *ab A*	-	13 *a A*	238 *a A*
Melon cv. Gallia					
11-01-93	6 *a B*	6 *a B*	-	0 *a A*	6 *a B*
05-03-93	18 *a A*	263 *ab A*	-	17 *a A*	95 *a A*
10-04-93	315 *a B*	862 *bc C*	-	63 *a A*	76 *a A*
15-05-93	495 *ab B*	1365 *c C*	-	133 *a A*	1190 *a C*
Tomato cv. Daniela					
04-10-93	53 *a A*	-	152 *a A*	200 *a A*	140 *a A*
10-11-93	82 *a A*	-	12 *a A*	1915 *a B*	1832 *a B*
20-12-93	66 *a A*	-	355 *a A*	3750 *a B*	4230 *b B*
30-01-94	71 *a A*	-	2650 *a B*	9120 *b C*	7200 *c C*
10-03-94	133 *a A*	-	12 100 *b B*	12 500 *b B*	9330 *d B*
20-04-94	111 *a A*	-	15 000 *b C*	12 790 *b C*	9350 *d B*

Data are means of four replications. Means in each column followed by the same small case letter and in each row followed by the same capital letter do not differ according to Newman and Keuls' test ($P \leq 0.05$).

Table 2. *Comparison of biological and chemical control: number of* Meloidogyne javanica *J2 extracted from 1 g root during three succesive vegetable crops.*

Sampling dates	*Pasteuria penetrans*	Itaconate	Cadusaphos	Methyl bromide	Control
Tomato cv. Eclaireur					
23-07-92	11 900 *b B*	9500 *c B*	-	25 *a A*	10 200 *e B*
23-09-92	15 400 *c B*	23 400 *d C*	-	97 *a A*	21 500 *g C*
09-11-92	58 *a A*	23 *a A*	-	39 *a A*	58 *a A*
Melon cv. Gallia					
05-03-93	322 *a B*	4185 *b C*		29 *a A*	7750 *d D*
15-05-93	160 *a A*	7900 *bc B*		250 *a A*	11 000 *e C*
Tomato cv. Daniela					
10-11-93	114 *a A*	-	89 *a A*	3600 *b C*	2050 *b B*
30-0194	50 *a A*	-	1750 *b B*	5100 *c C*	4300 *c C*
20-04-94	60 *a A*	-	18 500 *c B*	17 200 *d B*	19 100 *f B*

Data are means of four replications. Means in each column followed by the same small case letter and in each row followed by the same capital letter do not differ according to Newman and Keuls' test ($P \leq 0.05$).

Table 3. *Comparison of biological and chemical control: gall indices on the roots of three succesive vegetable crops.*

	Pasteuria penetrans	Itaconate	Cadusaphos	Methyl bromide	Control
Tomato cv. Eclaireur	4 *c B*	4.28 *a B*	-	0.56 *a A*	4.03 *a B*
Melon cv. Gallia	2.65 *b A*	4.05 *a B*	-	2.00 *b A*	4.25 *a B*
Tomato cv. Daniela	1.18 *a A*	-	1.50	3.63 *c B*	4.07 *a B*

Data are means of four replications with four roots in each replication. Means in each column followed by the same small case letter and in each row followed by the same capital letter do not differ according to Newman and Keuls' test ($P \leq 0.05$).

plot was planted with four rows of thirteen plants, nine plants from each of the two central rows were used for yield evaluation.

Nematode population in soil and roots, parasitism of second stage juveniles (J2) by *P. penetrans,* and gall indices were assessed at several sampling dates.

Samples were collected: *i*) before treatments, *ii*) at planting, and *iii*) every 40 days after planting. Nematodes were extracted by elutriation (Oostenbrink, 1960), and counted from 0.5 dm^3 soil samples made from four subsamples collected from each plot at 10 to 25 cm depth. Root nematodes were extracted by incubating samples for 10 days in a mist chamber (Seinhorst, 1950); the number of nematodes per g of root (fresh weight) was calculated.

Parasitism of J2 by *P. penetrans* was evaluated under the microscope (400 ×) by observing 50 J2 from each sample in *P. penetrans* infested plots. For the third crop, parasitism was also assessed in 0.5 dm^3 soil samples, dehydrated at 40°C, and mixed with a suspension of 20 000 J2 of *M. javanica* reared under laboratory conditions and incubated for 24 h before nematode extraction and parasitism evaluation.

At the end of each crop, four plants were randomly taken from each plot and evaluated for galling using a 0-5 scale (Kasumimoto *et al.*, 1993; Eddaoudi *et al.*, 1997).

Data were analysed by ANOVA with mean separation by the Newman and Keuls test ($P \leq 0.05$). Irrigation, fertilization, fungicide, and insecticide treatments were carried out following local practice.

Results

First crop (tomato)

Soil infestation of plots was homogenous before treatments (Table 1). Soil and root nematode popula-

Table 4. *Parasitism rate of* Meloidogyne javanica *by* Pasteuria penetrans *and number of spores per J2 in soil and roots on three succesive vegetable crops in* Pasteuria penetrans *treated plots.*

Crop Sampling date	Parasitism rate (%)		Spore number/J2	
	Soil	Roots	Soil	Roots
Tomato cv. Eclaireur				
26-06-92	27 *a*		1	
23-07-92	38 *ab*	72 *a*	2	1
19-08-92	79 *d*		2	
23-09-92	60 *c*	95 *a*	2	4
09-11-92	58 *c*	93 *a*	3	4.5
Melon cv. Gallia				
11-01-93	56 *c*		2	
05-03-93	50 *bc*	82 *a*	3	5
10-04-93	80 *d*		3	6
15-05-93	92 *d*	100 *a*	2	5
Tomato cv. Daniela				
04-10-93	100 *d*		5	
10-11-93	98 *d*	100 *a*	8	11
20-12-93	99 *d*		7	
30-01-94	100 *d*	100 *a*	10	15
10-03-94	99 *d*		9	
20-04-94	100 *d*	100 *a*	10	12

Data are means of four replications. Means in each column followed by the same letter do not differ according to Newman and Keuls' test ($P \leq 0.05$).

tions significantly ($P \leq 0.05$) increased during the first four sampling dates and dropped drastically at harvest (last sampling date) except in MB plots (Tables 1, 2). Soil and root recolonization by nematodes was low throughout the crop. The population fluctuation pattern in *P. penetrans* and ID treated plots was similar to that in the control plots.

Gall indices did not differ between *P. penetrans*, ID, and control treatments (Table 3). Roots in MB treated plots were white and had few or no galls or egg masses.

Table 5. *Comparison of biological and chemical control: yield (kg/plant) of three successive vegetable crops.*

	First crop	Second crop	Third crop
	Tomato cv. Eclaireur	Melon cv. Gallia	Tomato cv. Daniela
Pasteuria penetrans	0.71 *a*	1.32 *b*	4.38 *b*
Itaconate dimethyl	0.96 *b*	1.07 *a*	-
Cadusaphos	-	-	4.30 *b*
Methyl bromide	1.50 *c*	1.81 *c*	3.55 *a*
Control	0.75 *a*	1.00 *a*	3.61 *a*

Data are means of four replications with 18 plants in each replication. Means in each column followed by the same letter do not differ according to Newman and Keuls' test ($P \leq 0.05$).

Table 6. *Parasitism rate of* Meloidogyne javanica *by* Pasteuria penetrans *and number of spores per J2 in 0.5 dm^3 of soil in* Pasteuria penetrans *treated plots artificially infested with 20 000 J2.*

Sampling dates	Parasitism rate (%)	Spores number /J2 (mean ± SD)
04-10-93	95 *a*	5 ± 5.09
10-11-93	93 *a*	6 ± 3.31
20-12-93	96 *a*	4.5 ± 1.83
30-01-94	95 *a*	6 ± 4.21
10-03-94	95 *a*	4 ± 2.84
20-04-94	94 *a*	5 ± 3.69

Data are means of four replications. Means in each column followed by the same letter do not differ according to Newman and Keuls' test ($P \leq 0.05$).

Parasitism by *P. penetrans* was higher in roots than in soil. It first increased significantly in soil, then decreased slightly at harvest to 58 % (Table 4). In roots, percentage of parasitism remained stable.

Only ID and MB showed a significant yield increase over *P. penetrans* and control treatments. The yield in MB plots was higher than in ID plots (Table 5). Excessive vegetation was observed in MB plots. Yields in ID plots were low, but significantly higher than in *P. penetrans* and control plots. The yield in *P. penetrans* plots did not differ from the control.

Tomato mosaic virus (TMV) infection was recorded early in *P. penetrans* infested plots, then spread to all plots.

SECOND CROP (MELON)

Soil and root nematode populations were relatively low during the second crop as compared to the first

and the third crops. Populations were significantly lower at planting and increased in ID and control plots, but not in *P. penetrans* and MB plots (Tables 1, 2). *P. penetrans* and MB plots had lower gall indices than ID and control plots (Table 3).

Parasitism of soil J2 by *P. penetrans* spores increased from 56 % at planting to 92 % at harvest. In the roots, parasitism remained stable at more than 80 % throughout the crop (Table 4).

Yields were higher in MB and *P. penetrans* plots than in ID and control plots. There were no differences between these last two treatments. Yield in MB plots was greater than in *P. penetrans* plots (Table 5).

THIRD CROP (TOMATO)

Soil and root nematodes were low at planting and increased significantly from planting to harvest, except for *P. penetrans* plots where soil and root nematode populations remained low relative to the other treatments.

Soil and root nematode populations were suppressed during the first 3 months in the cadusaphos plots where soil population did not differ from that in *P. penetrans* plots. After the third month, nematode population build-up was higher than in the *P. penetrans* plots and lower than in control at mid crop. Cadusaphos, MB and control showed a similar trend in population build-up until harvest. Soil nematode populations in MB plots did not differ from the control except at harvest when MB and cadusaphos populations were the highest (Tables 1, 2).

After the first sampling, nematode root population in cadusaphos plots did not differ from that in *P. penetrans* plots (Table 2) and both populations were lower than in control plots; MB plots had the highest population. At the second sampling date, the nematode population had increased in cadusaphos treated soil; it was higher than *P. penetrans* population and lower than control and MB populations. At harvest, the two chemical treatment populations did not differ from the control population, whereas nematode root densities were the lowest in *P. penetrans* plots.

Gall indices did not differ between *P. penetrans* and cadusaphos plots and were significantly lower than in the MB and control plots (Table 3). In *P. penetrans* plots, galling occurred throughout the root systems, whereas in cadusaphos galls were aggregated on root tips.

Parasitism by *P. penetrans* in soil and roots increased to 100 %, with at least ten spores/J2 in average (Table 4). The assessment of *P. penetrans* parasitism on 20 000 J2 in 0.5 dm^3 soil revealed 95 % J2 parasitized with five spores per J2 in average. This rate of parasitism remained stable during the third crop (Table 6).

There were no differences in yield between *P. penetrans* and cadusaphos plots, although yield in *P. penetrans* plots was higher than yields in MB and control plots (Table 5).

Discussion

Methyl bromide has been widely used in Morocco for control of soil borne pests. In this study, it was effective during the first year on two successive crops (tomato and melon) but a high nematode infestation level was observed during the second year on the third crop (tomato). The cause of the excessive vegetative growth associated with methyl bromide treatment was not determined.

ID as used increased yield but had no effect on nematode populations. Treatment with cadusaphos at planting suppressed nematode infestation for 3 months and significantly increased yield.

On the first crop, *P. penetrans* had no effect on nematode populations and crop yield. However, an effect of *P. penetrans* was observed on nematode populations and gall indices during the second crop, which indicates that some time is needed to increase the bacteria population before effective nematode control occurs. Nematode populations and symptoms further declined during the third crop, which supports this conclusion. Our findings show that a first crop is needed to establish and increase spore density of *P. penetrans* in the soil before a second crop is protected from the nematodes. Another disadvantage is the risk of disease transmission due to the use of root powder as *P. penetrans* inoculum. TMV can be transmitted by plant residues (Blancard, 1988).

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