



Evaluation of Nematicidal Activity of Fluensulfone against *Meloidogyne incognita* in Bell Pepper Crop

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JACA and NZM managed the literature searches and edited the whole draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The evaluation of nematicidal activity of the non-fumigant nematicide (fluensulfone) was evaluated for the control of the root-knot nematode *Meloidogyne incognita* in bell pepper crop (*Capsicum annuum* L.) under field conditions. The experiment was set up under a randomized complete block design with four replications. Six treatments were assessed for control of *M. incognita*: four doses of fluensulfone, one of the nematicide oxamyl and a control without application of nematicides. Ten days before transplanting, nematicides were applied in a single application via irrigation systems. The lowest final population densities of *M. incognita* in bell pepper crop were recorded in plots treated with fluensulfone at the dose of 2.75 L·ha⁻¹, with an average of 25 juveniles. The higher percentage of efficacy was obtained with the application of fluensulfone at a dose of 2.75 L·ha⁻¹. Fluensulfone showed a more efficient nematicidal activity as compared with oxamyl, which is the most used nematicide in horticultural crops in Mexico. Our results indicated that fluensulfone can be used as an alternative nematicide for the control of *M. incognita* in horticultural crops.

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1. INTRODUCTION

The root-knot nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 is one of the major pest of bell pepper (*Capsicum annuum* L.) in México and in America. This nematode can cause production losses equivalent to about \$77 to 125 billion [1,2]. The most important plant-parasitic nematodes affecting bell pepper crop belongs to the genus *Meloidogyne* (Goldi, 1892) which is known as root-knot nematode. Reported yield losses of 24% to 28% in pepper worldwide due to infection by *M. incognita* [3].

Bell pepper is a relevant crop, its production has grown considerably in the last years, with a production of 4.5 million tons of dry and 36 million tons as fresh produce from 3.8 millions of hectares harvested in 2019. Fresh pepper is cultivated in 126 countries of the world in all the continents. The world's largest producer is China with over 18 million tons annually, followed by the Mexico with about 3.5 million tons [4,5].

Control of root-knot nematodes in horticultural crops system has been based primarily on broad spectrum nematicides. Methyl bromide, chloropicrin and 1,3-dichloropropene, and other pesticides including organophosphates and carbamates have been widely used to control plant parasitic nematodes.

Currently, the most widely used nematicides are organophosphates and carbamates, such as ethoprop, fenamiphos, fosthiazate and oxamyl, which are acetylcholine esterase inhibitors, thus these nematicides could be banned or restricted in the near future due to their toxicity and associated environmental impacts [6]. These nematicides are considered to be 'nematostatics' because at the recommended application doses they paralyse nematodes, or affect different aspects of nematode behavior, but they do not kill them. Often, nematodes that are paralysed by these substances recover after removal of the nematicide and become mobile and probably infective [7].

Fluensulfone [5-chloro-2-(3,4,4-trifluoro-but-3-ene-1-sulfonyl)-thiazole], which belongs to the fluoroalkenyl group, exhibits nematicidal activity and a far lower toxicity to vertebrates than organophosphate- or carbamate-based nematicides. The acute LD50 of fluensulfone to rats via oral administration is more than 500

mg·kg⁻¹, in contrast, the LD50 for popular nematicides such as aldicarb, fenamiphos, oxamyl, cadusafos, and fosthiazate are much lower; 0.5–1.5, 2–19, 5.4, 37.1 and 73 mg·kg⁻¹, for the nematicides mentioned respectively. Although some fluoroalkenyl compounds have been shown to have nematicidal activity, no nematicide based on this chemical group has been released so far in Mexico. In previous studies carried out by the present authors shown that, fluensulfone was true nematicidal, rather than nematostatic, activity. Fluensulfone is a contact nematicide that is applied via direct soil application and it is used for protecting a range of crop plants from plant parasitic nematodes damage and infestation. Studies have shown that fluensulfone has direct nematicidal activity against a number of plant parasitic nematodes (PPNs), including *Meloidogyne* spp., *Xiphinema index*, *Pratylenchus penetrans* and *P. thornei* [7,8].

Experiments in growth chambers, field microplots and commercial fields also indicated efficient nematode control by fluensulfone, even better control of nematodes than that of commercial nematicides widely used against root-knot nematodes.

In Mexico, only oxamyl is labeled for nematode control in bell pepper crop, therefore it is critical to provide more nematicide options to growers. Fluensulfone can be a good chemical alternative to control root-knot nematodes in commercial horticultural crops, but its efficacy has to be tested. For this reason, our objectives were (i) to determine the effects of fluensulfone on infection of bell pepper roots by *M. incognita* and the subsequent population development and (ii) to evaluate the biological effectiveness of fluensulfone against *M. incognita* in bell pepper crop under field conditions. The central hypothesis tested was that fluensulfone possesses nematicidal activity to provide control of *M. incognita*, thus reducing population development and improving the growth of bell pepper crop.

2. MATERIALS AND METHODS

2.1 Study Location and Site Description

The research was carried out at Chapingo Agricultural Experiment Station, the soil is natural infested with *M. incognita*. The Experiment Station is in Texcoco, Mexico. The soil within the

farm was a sandy clay loam (51% sand, 25% clay, 24% silt), with pH of 6.9. The annual rainfall average in the area is 645 mm, temperature ranges from 5 to 25°C.

2.2 Experimental Field and Experimental Design

A randomized complete block design was used for the experiment. The experimental units consisted of rows of 10 m long and 0.6 m wide, equivalent to 9 m². The experimental design consisted of 6 treatments (4 fluensulfone doses + one oxamyl dose + control without any nematicide) × 4 replicates = 24 treatments, so the experimental plot was constituted of 24 experimental units giving a total of 219 m². Specifically, the five treatments that were applied to the experimental units were: 1) Nimitz (fluensulfone) at four doses (2.0, 2.25, 2.5, 2.75 L·ha⁻¹), 2), vydate L (oxamyl) at a dose of 3 L·ha⁻¹, and 3) control (without application of nematicides). Nematicides were applied twelve days prior to transplanting via drip irrigation systems.

During the experiment the plants were kept until harvest, fertilized via irrigation system with the following dose of nutrients: 215 N, 77 P₂O₅, 350 K₂O at dosages of Kg·ha⁻¹, and watered every day to field capacity via drip irrigation system. Control of pests, diseases, and weeds were made according to the recommendations of local growers, avoiding the application of products with nematicide action. The bell pepper variety used for the experiment was Orion.

2.3 Response Variables

2.3.1 Initial and final population densities

Ten soil samples at a depth of 15-20 cm from each plot were collected to form a composite sample of about one kilogram (four samples per treatment). All were processed to extract and quantify the number of juveniles (J2) of *M. incognita* by Cobb's method [9]. The initial assessment was made before treatment application and the final evaluation was made 60 days after transplantation of bell pepper seedlings.

2.3.2 Galling index and percentage of galled roots

Ten plants from each experimental unit (40 plants per treatment) were extracted using a

shovel, the root system was observed to calculate the galling index, which was determined by comparison with a visual scale of five degrees, where 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 galls [10]. The galling index is a good methodology to assess the degree of infection of species of root knot nematodes in the roots of their hosts and to measure the degree of damage caused to the crop or severity [10]. The percentage of galled roots per plant was calculated by using the following formula:

$$PGR = \left(\frac{NAR}{TR} \right) * 100$$

Where: PGR= Percentage of Galled Root; NRA= Number of Galled Roots; TR= Total Roots.

2.3.3 Efficacy of nematicides against *M. incognita*

Once the percentage of root gall was calculated, the next formula was applied to determine the efficacy of nematicide treatments [11]:

$$TE = \frac{GC - gt}{GC} * 100$$

Where TE: Treatment efficacy; GC: Percentage of galled root in the control; gt: Percentage of galled root in the treatments.

2.3.4 Plant parameters

The following plant parameters were measured; plant height (40 per treatment), and fresh root weight (20 per treatment).

2.4 Statistical Analysis

The experiment was repeated twice. All the data were subjected to Analysis of Variance (ANOVA) using SAS statistical software version 9.4 [12]. The means were compared by Tukey's protected least significant difference test at ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Initial and Final Population Densities

The initial population densities of *M. incognita* were low and uniform, the population did not differ across plots (i.e., not significantly different). Number of nematodes ranged from 2.5 to 6 individuals (J2) in 100 g of bell pepper cultivated soil (Table 1). This finding was expected since

the initial nematode population was quantified prior to planting, and at this point in time there is an absence of host plants. *M. incognita* is an endoparasite, which means that it needs a suitable host plant to complete its life cycle, in absence of crops or suitable hosts, nematode population tend to be lower [13].

Final population densities of *M. incognita* ranged from 25 to 75.25 J2 in 100 g of soil (Table 1) and were significantly different ($P \leq 0.05$) according to treatments. Plots treated with fluensulfone at different doses had significantly a smaller number of nematodes as compared with control plots and with plots treated with oxamyl (Table 1). The lowest numbers of nematodes were found in plots treated with fluensulfone at doses of 2.75 and 2.5 L·ha⁻¹. In these plots, 25 and 26.75 J2 were observed respectively. The results evidenced that fluensulfone exerted a detrimental effect on nematode populations, preventing their increase in soil to levels that could generate significant damage to crop, thereby giving good protection during the susceptible crop stage. Also, we can infer that fluensulfone produced a reduction in reproduction rate on females, which is a detrimental effect on the nematode population.

An increase in final population of *M. incognita* in soils was expected due to the presence of suitable hosts (bell pepper plants) and favorable conditions of humidity and temperature [14].

Fluensulfone could interfere with these neurotransmitter and neuromodulator signalling pathways to reduce egg laying. Alternatively, fluensulfone may reduce egg laying through impairment of the contraction of the vulval muscles. The inhibition of feeding by fluensulfone could also bring about a reduction in egg laying, as it has been observed that adult hermaphrodites can enter a reproductive diapause under starvation conditions during which egg laying is substantially reduced [15]. Fluensulfone had several effects on locomotion that varied with concentration, and the concentration- and time-dependence of these effects differed in the presence and absence of a food source and differed between a solid substrate and liquid media.

Our findings agreed with other studies, which evidenced that fluensulfone applied to soil prior to transplantation had a detrimental effect on populations of *M. incognita* and *Nacobbus aberrans* in tomato and tobacco plantations [16].

Oxamyl provided lower protection as compared with fluensulfone. Oxamyl belongs to the chemical group of carbamates, which act as nematostatic at low concentrations and short exposure periods. This nematicide can paralyze plant-parasitic nematodes, affecting some aspects of their behavior, such as orientation and hatching [17,18,19,20]. Finally, populations of *M. incognita* in control plots (without any nematicide applied) increased normally because these organisms were not exposed to detrimental factors (no nematicides) and found susceptible plant hosts as food source.

3.2 Percentage of Galled Roots and Gall Index

The percentage of galled roots significantly differed ($P \leq 0.05$) according to treatments applied (Table 2). Plants grown in control plots had the highest rate of infection (58.43%), meanwhile plants grown in plots treated with fluensulfone at doses of 2.5 and 2.75 L·ha⁻¹ showed roots with reduced galling percentage as compared with controls (i.e., 11.50 and 9.30% respectively) (Table 2). Differences in percentage of galled roots did not translate into differences in gall index, which did not differ in plants according to pesticide treatments. The values of gall index ranged from 1.98 to 4.60, the lowest gall indexes were found in bell pepper plants grown in plots treated with fluensulfone at doses of 2.5 and 2.75 L·ha⁻¹ (2.03 and 1.98 respectively); whereas plants grown in control plots without any application of pesticides showed the highest gall indexes (4.60) (Table 2).

The low gall indexes, together with the reduction in nematode numbers in soil and roots indicated an effective control of *M. incognita* in bell pepper plants by fluensulfone. Fluensulfone was found to delay and inhibit *Caenorhabditis elegans* development. Fluensulfone also inhibited *C. elegans* egg laying and the subsequent hatching of these eggs.

In pot studies with *M. javanica*, fluensulfone has been shown to prevent root galling. Root galls are only formed when *Meloidogyne spp.* have entered the host and induced the formation of nurse cells [8]. This indicates that fluensulfone either prevent the juveniles from invading the host plant, prevents the induction of nurse cells or prevents the maturation and development of PPN inside the host. Furthermore, fluensulfone was shown to prevent the production of eggs in tomato roots exposed to *M. javanica* [7,8].

Table 1. Initial (Ip) and final (Fp) population densities of *M. incognita* (J2·100 g⁻¹soil) found in plots treated with fluensulfone at different doses and oxamyl (commercial control). Plots with no application of nematicides acted as absolute controls. Means followed by the same letters in each column are not significantly different ($P \leq 0.05$)

Treatment	Nematicide	Dosage (L·ha ⁻¹)	Ip	Fp
1	Oxamyl	3.0	6.00 ^a	40.5 ^b
2	Fluensulfone	2.0	4.50 ^a	31.5 ^c
3	Fluensulfone	2.25	5.25 ^a	27.0 ^c
4	Fluensulfone	2.5	3.50 ^a	26.75 ^c
5	Fluensulfone	2.75	4.00 ^a	25.0 ^c
6	Control	-----	2.50 ^a	75.25 ^a

Table 2. Percentage of Galled Roots (PGR) and Gall Index (GI) observed in bell pepper plants grown in plots treated with fluensulfone at different doses. oxamyl acted as commercial control. plots with no application of nematicides acted as absolute controls. Means followed by the same letters in each column are not significantly different ($P \leq 0.05$)

Treatment	Nematicide	Dosage (L·ha ⁻¹)	PGR	GI
1	Oxamyl	3.0	16.00b	2.65a
2	Fluensulfone	2.0	13.00b	2.28a
3	Fluensulfone	2.25	14.50b	2.18a
4	Fluensulfone	2.5	11.50b	2.03a
5	Fluensulfone	2.75	9.30b	1.98a
6	Control	-----	58.43a	4.60a

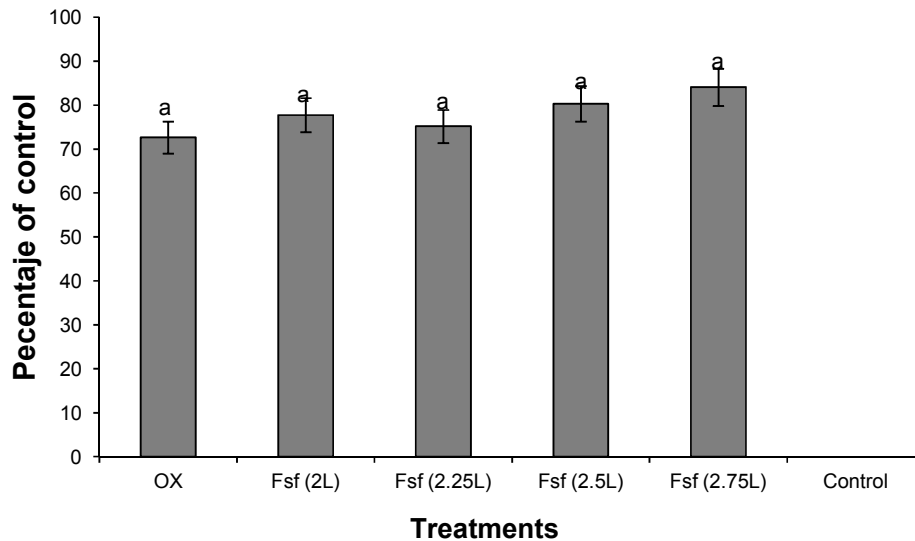


Fig. 1. Percentages of efficacy of the nematicide fluensulfone (Fsf) at different doses, and oxamyl (OX) against *M. incognita* in bell pepper plants. Means followed by the same letters in each bar are not significantly different ($P \leq 0.05$)

3.3 Efficacy of Nematicides against *M. incognita*

The efficacy of nematicides did not significantly differ across treatments, but the best control of *M. incognita* was obtained in plots treated with fluensulfone at doses of 2.5 and 2.75 L·ha⁻¹; in

these plots percentages of efficacy were 80.32% and 84.08% respectively (Fig. 1).

Nematicide effect of fluensulfone against the root-knot nematodes *Meloidogyne* spp. have been showed in different crops under experimental conditions, including pot and field

experiments using tomato and pepper and in a tomato-cucumber double cropping system, as well as in lima bean fields [7,21].

The results agreed with other studies that indicate an efficient control of *M. incognita* by fluensulfone, which is explained in part to the irreversible nematicidal effect of the nematicide [7,8,15].

Fluensulfone provided better control of *M. incognita* as compared with oxamyl, which is the most used nematicide in horticultural crops, and these results agreed with previous reports [7,8].

Fluensulfone applied to bell pepper plants after inoculation with *M. incognita* reduce plant parasitic nematodes damage after root invasion [7,8]. The prevention of root damage by soil drenching with fluensulfone declined with time after the initial inoculation with *M. incognita*. This suggests that fluensulfone has activity against *Meloidogyne* spp. both pre- and post-invasion. As the ability of fluensulfone to reduce damage declines with time after root invasion this suggests that earlier developmental stages are more susceptible to fluensulfone or that fluensulfone inhibits juvenile motility in the root, feeding site formation or stylet behaviour.

Sublethal fluensulfone exposure impairs the ability of some PPNs to invade the host plant. This may be due to diminished host-finding capability. These observations also serve to highlight the difference between the nematostatic organophosphates, from which PPNs can rapidly recover, and fluensulfone, which has longer lasting effects.

It was observed in these species that fluensulfone exposure resulted in a paralysis from which the nematode failed to recover and this paralysis was characterised by a rod-shaped body posture. This suggests that *M. javanica*

cannot recover after absorbing a lethal dose of fluensulfone and that there is delay between absorbing a lethal dose and the death of the nematode [8]. This indicates a novel mode of action for fluensulfone relative to other currently used nematicides. Plant parasitic nematodes can also recover after treatment with organophosphates and carbamates yet do not recover from fluensulfone treatment, which further implicates a novel mode of action relative to current nematicides.

3.4 Growth Parameters

Plant height showed no statistical differences ($P \leq 0.05$) according to pesticide treatments. Mean values for plant height ranged between 38.21 and 61.49 cm (Table 3), however the highest plants were found in plots treated with fluensulfone at the dose of 2.75 L·ha⁻¹ and the smallest plants were found in control plots.

Fresh root weight showed no statistical significance ($P \leq 0.05$) among treatments, with average values ranged between 27.43 and 39.75 g per plant (Table 3). The greater weight of roots was obtained from plants grown in plots treated with fluensulfone at a dose of 2.75 L·ha⁻¹.

Despite our results indicated significant differences in parameters associated to nematode infection (i.e., population density of J2 in soil, percentage of galled roots and gall index) according to nematicide treatments, these findings did not translate into significant differences in plant parameters. We could attribute this phenomenon to fertilization of bell pepper plants, which was adequate. Fertilizer application could partially, offset *M. incognita* damage in bell pepper crop by stimulating plant development, as observed in previous studies with different crops. These studies have evidenced that a good nutrition help plants to counteract damage produced by

Table 3. Plant Height (PH) and Fresh Root Weight (FRW) observed in bell pepper plants grown in plots treated with fluensulfone at different doses, and oxamyl (commercial control). plants with no application of nematicides acted as absolute controls. Means followed by the same letters in each column are not significantly different ($P \leq 0.05$)

Treatment	Nematicide	Dosage (L·ha ⁻¹)	PH (cm)	FRW (g)
1	Oxamil	3	43.13a	29.32a
2	Fluensulfone	2	59.21a	36.12a
3	Fluensulfone	2.25	53.67a	33.14a
4	Fluensulfone	2.5	59.42a	37.21a
5	Fluensulfone	2.75	61.49a	39.75a
6	Control	-----	38.21a	27.43a

plant-parasitic nematodes [22,23]. On the other hand, nitrogen in ammonium form, present in the fertilizer applied to crops, has a negative effect on nematodes due to its plasmolytic effect around the point at which it is applied to the soil [22].

4. CONCLUSION

The present research revealed that application of fluensulfone under experimental conditions in bell pepper crop provided a good control against root-knot nematodes. Based on these findings the lowest nematode population were obtained in the plots treated with fluensulfone at doses of 2.75 L ha⁻¹. Therefore, fluensulfone can be suggested as an alternative nematicide for the control of *M. incognita* on bell pepper crop. However further evaluation of this nematicide must be carried out in different crops and evaluate its nematicidal properties against other plant-parasitic nematodes under commercial field conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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