

Original Research Article

Nematicidal efficacy of fluensulfone against false root-knot nematode (*Nacobbus aberrans*) in cucumber crop under field conditions

ABSTRACT

The present study was carried out to compare the efficacy of a nematicide of new generation for the control of false root-knot nematode (*Nacobbus aberrans*) in cucumber crop (*Cucumis sativus* L.) under field conditions. The experiment was set up under a randomized complete block design (RCBD) with four replications. Six treatments were assessed for control of *N. aberrans*: four doses of fluensulfone, one of the nematicide oxamyl and a control with no application of nematicides. Ten days before transplanting, nematicides were applied in a single application via irrigation systems. Higher control under field conditions was obtained with the application of fluensulfone at a rate of 2.75 L·ha⁻¹. The lowest final population densities of *N. aberrans* in cucumber crop were recorded in the plots treated with fluensulfone at the rate of 2.75 L·ha⁻¹, with an average of 6.25 juveniles.

Keywords: cucumber, efficacy, fluensulfone, nematicide, oxamyl.

1. INTRODUCTION

The false root knot nematode (*Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944) is a plant-parasitic nematode native to the Americas [1]; it has quarantine importance [2], and has been found in association with numerous crops and native plants in temperate and subtropical areas of North and South America. This nematode is traditionally controlled through extensive use of fumigants, predominately methyl bromide. However, the use of methyl bromide is now restricted in México and many other countries due to its ozone-depleting properties [3]. Other types of nematicides such as carbamates (aldicarb) and organophosphates (fosthiazate) have been used in place for methyl bromide, but these could be banned or restricted in the near future due to their toxicity and associated environmental impacts [4]. For these reasons, the production of nematicides that are effective against plant-parasitic nematodes, little toxic and environmentally safer is necessary. Currently, there are some new nematicides but their nematicidal properties and environmental effects have to be tested before release to the market. One example is fluensulfone (5-chloro-2-(3,4,4-trifluorobut-3-enylsulfonyl)-1,3-thiazole) that belongs to the fluoroalkenyl thioether group. Fluensulfone has good nematicidal properties, it is effective against a wide number of plant-parasitic nematodes including *N. aberrans* [5, 6], lacks many of the drawbacks of other chemical nematicides, as it is relatively low toxic to non-target organisms [7]. These properties make fluensulfone a good chemical alternative to traditional nematicides utilized for control of plant-parasitic nematodes, but such properties have to be confirmed before its intensive use.

Nacobbus aberrans is one of the main plant-parasitic nematodes that affect cucumber production (*Cucumis sativus* L.); the nematode causes alterations in the root system and losses in production [1]. In Mexico, cucumber production is important since it is one of the main horticultural crops, being this country the second largest exporter worldwide, and the first supplier to the US market [8]. Additionally, cucumber production represents an alternative to diversify crop systems locally, meet the demand of domestic markets, and supply nutritional requirements of humans, as it is rich in vitamins A, B, C and minerals [9, 10]. The cucumber cultivated area in the country is currently about 16500 ha in fields, representing a production of 188491t. *N. aberrans* causes severe damage to cucumber production in Mexico; it has been found in the states of Coahuila, Guanajuato, Hidalgo, Mexico, Morelos, Puebla, Oaxaca, San Luis Potosí, Tlaxcala and Zacatecas [11]. However, in most cucumber production states production losses associated with *N. aberrans* have not been estimated. Reports from other crops (i.e., tomato, potato) indicate up to 100 % of root damage due to *N. aberrans*, and yield losses ranging from 50 to 90 %

[12,13], this information highlights the level of damage that this nematode can produce to crops and the necessity of effective measures for its control.

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

2. MATERIAL AND METHODS

2.1 Study location and site description

The research was carried out at a commercial cucumber farm infested with *N. aberrans*. The farm was located in Cojumatlán de Régules, Michoacán, in Mexico.

2.2 Experimental field

The experimental units consisted of rows of 15 m long and 0.8 m wide, equivalent to 12 m², so the experimental plot was constituted of 24 experimental units giving a total of 288 m². The plants were kept until harvest, fertilized (N-P-K (13-40-13)), and watered every day. Control of pests, diseases, and weeds was made according to the recommendations of local growers, avoiding the application of products with nematicide action. The cucumber variety used for the experiment was Cross Country F1.

2.3 Treatments and Experimental Design

Six treatments were applied to the experimental units: 1) 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). A randomized complete block design was used for the experiment. Nematicides were applied ten days prior to transplanting via drip irrigation systems.

2.4 Response variables

2.4.1 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 *N. aberrans* by Cobb's method [14]. The initial assessment was made before treatments application and the final evaluation was made 63 days after transplantation.

2.4.2 Gall index and percentage of root gall

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 [15]. 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 [16].

The percentage of root gall per plant was calculated by using the following formula:

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

Where: PGR= Percentage of root gall; NRA= Number of galled roots; TR= Total roots

2.4.3 Efficacy of nematicides against *N. aberrans*

Once the percentage of root gall was calculated, the next formula was applied to determine the efficacy of nematicide treatments [17]:

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

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

2.4.4 Plant parameters

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

2.5 Statistical analysis

The experiment was repeated twice. All the data were subjected to Analysis of Variance (ANOVA) using SAS statistical software version 9.2 [18]. The means were compared by Tukey's protected least significant difference test at (P= 0.05).

RESULTS AND DISCUSSION

3.1 Initial and final population densities

The initial populations of *N. aberrans* showed no statistical significance (P= 0.05) between treatments, with values ranging from 1.75 to 2.25 juveniles (J2) in 100 g of soil (Table 1). The initial nematode populations were low and uniform. This is explained by the absence of host plants and biology of this species due to their endoparasitic way of living and feeding, therefore in absence of crops or suitable hosts nematode population tend to be lower [19].

Final populations of *N. aberrans* showed differences according to treatments. Fluensulfone treatments significantly differed oxamyl and negative controls (P= 0.05), with final population densities ranged from 6.25 to 8.75 J2 in 100 g of soil (Table 1). The lowest values of final populations were found in fluensulfone treatment at dose of 2.25 L·ha⁻¹. The results from these studies show that fluensulfone soil treatments applied at prior to planting can reduce the infection in cucumber roots by *N. aberrans*.

Nematode reproduction is affected by several factors such as temperature, availability of host plants, agricultural practices, among others [20]; therefore nematodes population in control plots had higher population increases due to absence of limiting factors. On the other hand, nematodes population were lower in plots treated with chemicals which significantly reduced reproduction of females nematodes due exposed to lethal and sublethal doses of pesticides. Nematodes that survived after nematicide application could reproduce, but may hatching could be inhibited due fluensulfone [21].

Fluesulfone inhibited *Caenorhabditis elegans* egg-laying, larval development and feeding in addition to its effects on motility and egg hatching. One might predict therefore, that fluensulfone would have a similar range of actions against plant parasitic nematodes including the false root-knot nematode [22].

In an *in vitro* experiment [22] said that the exposure time of one hour of *C. elegans* to fluensulfone had a high proportion of eggs that were subsequently laid failed to hatch. Inspection of the un-hatched eggs revealed that they had been fertilized, therefore the nematicidal effect in utero occurs post-fertilization. This is a remarkable effect and is consistent with fluensulfone gaining rapid access to the internal structures of the nematode which then elicit prolonged detrimental effects not only on the adult

nematodes but also on their progeny. Such an effect in plant parasitic nematodes could conceivably contribute to the reported nematicidal effects of fluensulfone following systemic application [21]. Other systemic nematicides have been reported to have inhibitory effects on egg hatching for various species of plant parasitic nematodes [23, 24, 25].

The suggestion that fluensulfone inhibits feeding may be related to the fact that it induces larval arrest: There is a known link between feeding and arrested development. Thus, juveniles nematodes can enter a diapause state when embryos hatch where food is scarce, during which they do not moult to the next stage until food becomes more abundant [26].

Whilst juveniles diapause can continue for several days, mortality increases as the length of starvation increases. It is possible that larvae arrest as they cannot feed, even in the presence of abundant food. Fluensulfone could also inhibit development through interfering with moulting process. Whilst other chemicals have been shown to induce juvenil and embryonic arrest, the mechanisms by which this arrest is induced are not known [26]. Inhibition of mitochondrial function is one mechanism through which fluensulfone could inhibit egg hatching in several nematodes species [22].

According to those previous in vitro experiments, fluensulfone inhibit nematode hatching and movement, therefore exerting a detrimental effect on several plant parasitic nematodes including populations of *N. aberrans*, thus preventing their increase to levels that could generate significant crop damage, thereby giving good protection to susceptible crops. Other works have evidenced a significant decrease in populations of *Meloidogyne arenaria* and *N. aberrans* by fluensulfone applications in tobacco and tomato; respectively in these experiments fluensulfone was especially effective during pretransplant applications [6]. Reducing the size of potential inoculum, with respect to the number of J2 that infect a host crop is one of the main principles underlying soil treatments with nematicides, rationale being the direct relationship between the infection activities of the juveniles J2 and crop yield.

There was some suppression of *N. aberrans* by oxamyl, however this nematicide provided lower protection as compared with fluensulfone (13.75 J2 in 100 g of soil). Oxamyl belongs to the chemical class of carbamates, which act as nematostatic at very low concentrations and short exposure periods [27]; this substance paralyzes nematodes, affecting some aspects of their behavior (i.e., orientation, hatching) but it does not kill the nematodes, allowing the population recovery after some period of time [5].

Final populations of *N. aberrans* in the control plots increased as expected. This treatment displayed the highest numbers of nematodes (30.5 J2 in 100 g of soil) due to lack of control measure.

Table 1. Effect of nematicides on populations of *N. aberrans* in cucumber crop.

Treatment	Nematicide	Dosage (L·ha ⁻¹)	Ip	Fp
1	Oxamyl	3	2.00 ^a	13.75 ^{ab}
2	Fluensulfone	2	2.50 ^a	7.50 ^b
3	Fluensulfone	2.25	2.25 ^a	6.25 ^b
4	Fluensulfone	2.5	2.00 ^a	7.00 ^b
5	Fluensulfone	2.75	2.00 ^a	8.75 ^b
6	Control	-----	2.50 ^a	30.50 ^a

*Means followed by same letters in the same column are non-significant different (P=0.05).

Ip =initial population, Fp = final population.

3.2 Percentage of root galling index

Galling index values in cucumber plants showed statistical differences according to treatments (P= 0.05). The lowest values were found in fluensulfone treatments with values ranging from 2.1 to 2.5 (Table 2). And from these treatments the lowest GI was obtained at a dose of 2.25 L·ha⁻¹. The highest values were found in the control plants as was expected.

Table 2. Percentage of root gall and galling index on cucumber plants.

Treatment	Treatment	Dosage (L·ha ⁻¹)	PRG	GI
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1	Oxamyl	3	24.00 ^b	3.4 ^{ab}
2	Fluensulfone	2	12.00 ^b	2.2 ^b
3	Fluensulfone	2.25	11.50 ^b	2.1 ^b
4	Fluensulfone	2.5	13.00 ^b	2.3 ^b
5	Fluensulfone	2.75	15.50 ^b	2.5 ^b
6	Control	-----	61.00 ^a	4.1 ^a

*Means followed by same letters in the same column are non-significant different (P=0.05).

PRG= Percentage of root gall; GI= Galling index.

The low root gall indexes observed in all chemical treatments together with the reduction in nematode numbers in soil and roots indicated effective control of the false root- knot nematode.

3.3 Efficacy of nematicides against *N. aberrans*

The best control against *N. aberrans* was obtained in the plots treated with fluensulfone at doses of 2.25 and 2 L·ha⁻¹, with values of 81.15% and 80.33 % percentage of control, respectively (Figure 1). Other researchers found better control of root-knot nematodes in plots treated with fluensulfone due to its irreversible nematicidal effect [5, 28].

Fluensulfone provided better protection and control of *N. aberrans* as compared with oxamyl, which is the most used nematicide in horticultural crops. These results agreed with previous reports [29].

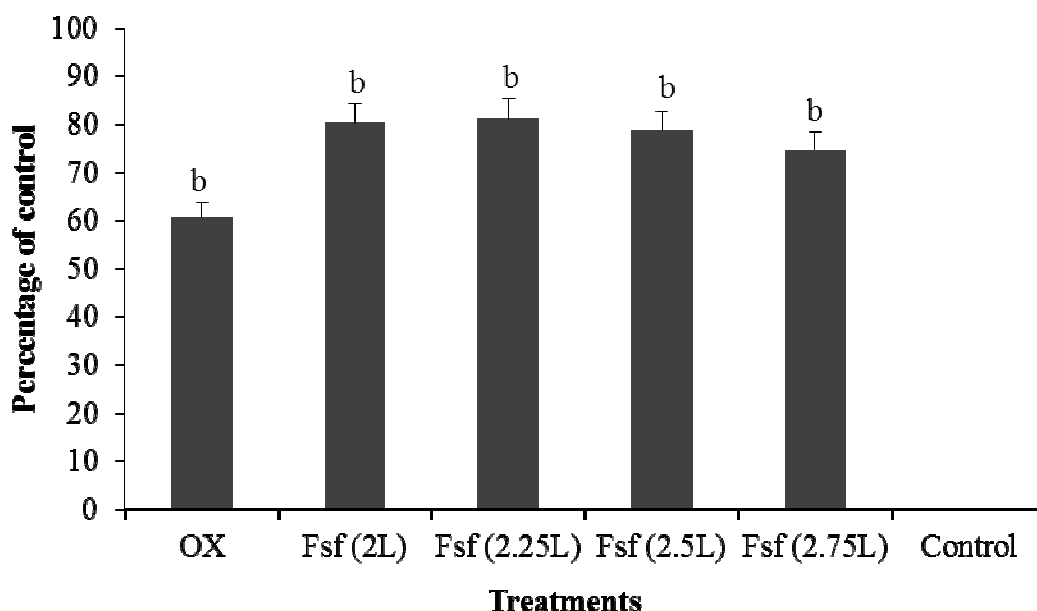


Figure 1: Nematicide effect of fluensulfone against *N. aberrans* on cucumber plants.

3.4 Growth parameters

Vine length showed no statistical differences (P= 0.05) among treatments, with values ranging between 97.80 and 113.40 cm (Table 3). However, the longest plants were found in plots treated with fluensulfone at the dose of 2.0 L·ha⁻¹. Application of fluensulfone and oxamyl increased biomass production of different crops [5, 28].

214 Fresh root weight showed no statistical significance ($P= 0.05$), with averages values of 7.18 to 8.21 g per
 215 plant (Table 3). The greater weight of roots was obtained from the plots treated with oxamyl at a dose of 3
 216 $L \cdot ha^{-1}$.
 217

218 **Table 3. Vine length and fresh root weight.**

Treatment	Nematicide	Dosage ($L \cdot ha^{-1}$)	VL		FRW	
1	Oxamyl	3	110.60	A	8.210	A
2	Fluensulfone	2	113.40	A	7.250	A
3	Fluensulfone	2.25	108.30	A	7.680	A
4	Fluensulfone	2.5	102.80	A	7.660	A
5	Fluensulfone	2.75	97.80	A	7.180	A
6	Control	-----	100.70	A	7.390	A

219 Means followed by the same letters in each column are not significantly different ($P= 0.05$). VL= Vine
 220 length, FRW= fresh root weight
 221

222 Applying fertilizer could partially, offset nematode-induced damage in cucumber crop by stimulating plant
 223 development. Similar results were obtained in cotton, where the plant parasitic nematode *Rotylenchulus*
 224 *reniformis* Linford and Oliveira did not affect plant growth in the presence of high levels of available
 225 potassium [30]. Similar responses have also been observed for micronutrients [31]. In another study
 226 carried out under greenhouse conditions have shown that applying macronutrients to sugarcane reduces
 227 the severity of root damage caused by *Meloidogyne* spp., allowing the plant to develop normally [31].
 228 Nitrogen in ammonium form, present in the fertilizer applied to cucumber crop, has a negative effect to
 229 nematodes due to its plasmolytic effect around the point at which it is applied to the soil. Nevertheless,
 230 other indirect mechanisms, such as an increase in microbiota antagonistic to nematodes, may also have
 231 a significant effect. Many fungi in the soil prefer nitrogen in ammonium form, which means that ammonia-
 232 producing materials could encourage proliferation of nematode parasitic fungi. Because of nematicidal
 233 effect of ammonium nitrogen, urea has also been the subject of many studies, since it is rapidly converted
 234 into ammonia by the action of urease in the soil [31]. In other hand plants with high levels of phosphorus,
 235 release fewer root exudates and therefore are less attractive to plant parasitic nematodes therefore
 236 decreasing the incidence of the diseases. Furthermore, plants become more resistant when supplied with
 237 sufficient quantities of phosphorus as a result of increases in protein synthesis, cell activity and
 238 production of polyphenols, peroxidase and ammonia [31]. The results shown that fluensulfone application
 239 via drip irrigation would be a chemical alternative control for *N. aberrans*. Additional experiments are
 240 necessary, mainly in other horticultural crops.
 241

242 243 4. CONCLUSION

244
 245 The present study revealed that application of fluensulfone under experimental conditions in cucumber
 246 crop provided a good control against the false root-knot nematode. Based on these findings the lowest
 247 nematode populations were obtained in the plots treated with fluensulfone at doses of 2.75 $L \cdot ha^{-1}$.
 248 Therefore fluensulfone can be suggested as an alternative nematicide for the control of *N. aberrans* on
 249 cucumber crops. Further evaluation of this nematicide should be carried out in different crops and
 250 evaluate its nematicidal properties against other plant-parasitic nematodes under commercial field
 251 conditions.

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