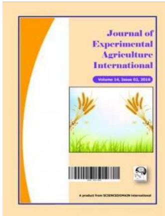

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Nematicidal Efficacy of Fluensulfone against False Root-knot Nematode (*Nacobbus aberrans*) in Cucumber Crop under Field Conditions

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Authors' contributions

This work was carried out in collaboration between both authors. Author JACA designed the experiment, wrote the protocol, collected and analyzed data and wrote the manuscript. Author MOA review the experimental design, analyzed data and contributed to wrote the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to compare the efficacy of a nematicide of new generation for the control of the 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 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 dose of 2.75 $L \cdot ha^{-1}$. The lowest final population densities of *N. aberrans* in cucumber crop were recorded in the plots treated with fluensulfone at the dose of 2.25 $L \cdot ha^{-1}$, with an average of 6.25 juveniles, and

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the lowest galling index was observed in plots treated with fluensulfone at the same dose, with a galling index of 2.1. The results indicated that application of fluensulfone to cucumber crop can provide good control of *N. aberrans*.

Keywords: Cucumber; efficacy; fluensulfone; nematicide; oxamyl.

1. INTRODUCTION

The false root knot nematode (*Nacobbus aberrans* [1] is a plant-parasitic nematode native to the Americas [2]; it has quarantine importance [3], 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 Mexico and many other countries due to its ozone-depleting properties [4]. Other types of nematicides such a 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 [5]. For these reasons, the production of nematicides that are effective against plant-parasitic nematodes, less 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 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* [6,7], and lacks many of the drawbacks of other chemical nematicides, as it is relatively less toxic to non-target organisms [8]. 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 [2]. In Mexico, cucumber production is important since it is one of the main horticultural crops, this country being the second largest exporter worldwide, and the first supplier to the US market [9]. 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 [10,11]. The cucumber cultivated area in the country is currently about 16500 ha in fields, representing a production of 188491t. *Nacobbus 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 [12]. 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% [13,1], 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 nematicidal activity to provide control of *N. aberrans*, thus reducing population development and improving the growth of cucumber crop.

2. MATERIALS 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 Regules, Michoacán, in Mexico. The soil within the farm was a loamy sand (85% sand, 11% silt, 4% clay), with pH of 6.2. The annual rainfall in the area ranges between 700 and 1000 mm, temperature ranges from 16 to 20℃, with 58% of relative humidity.

2.2 Experimental Field and Experimental Design

A randomized complete block design was used for the experiment. The experimental units consisted of rows of 15 m long and 0.8 m wide, equivalent to 12 m^2. The experimental design consisted of 6 treatments (4 fluensulfone doses + one oxamyl dose + control with no nematicide) × 4 replicates = 24 treatments, so the experimental plot was constituted of 24 experimental units giving a total of 288 m^2. 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 $L \cdot ha^{-1}$), 2), vydate L (oxamyl) at a dose of 3 $L \cdot ha^{-1}$, and 3) control (without application of nematicides). Nematicides were applied ten days prior to transplanting via drip irrigation systems.

During the experiment the plants were kept until harvest, fertilized via irrigation system with nitrofoska (N-P-K (13-40-13)) at a dosage of 25 $Kg \cdot ha^{-1}$, and watered every day to field capacity via drip irrigation system. 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 variety.

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 *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.

2.3.2 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 [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 galled roots per plant was calculated by using the following formula:

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

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

2.3.3 Efficacy of nematicides against *Nacobbus 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.

2.3.4 Plant parameters

The following plant parameters were measured; vine length (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 9.2 [18]. The means were compared by Tukey's protected least significant difference test at (P= 0.05).

3. RESULTS AND DISCUSSION

3.1 Initial and Final Population Densities

The initial populations of *N. aberrans* in the experimental field ranged from 2.00 to 2.50 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 from oxamyl and from controls (P= 0.05), with final population densities in fluensulfone treatments ranging 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 $L\cdot ha^{-1}$. Our results suggest that fluensulfone applied to soil 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 nematode population in control plots had higher population densities since there is absence of limiting factors. On the other hand, nematode populations were lower in plots treated with chemicals; these substances significantly reduced the reproduction of nematode females due to exposure to lethal and sublethal doses of pesticides. Nematodes that survived after nematicide application could reproduce, but hatching could be inhibited by exposure to fluensulfone [21]. Inhibition of mitochondrial function is one mechanism through which fluensulfone could inhibit egg hatching in several nematode species [22]. An advantage of fluensulfone is that negatively affects other stages and activities of nematodes in addition to egg hatching; for example, egg-laying, larval development, feeding, moulting, and motility [22]. Another advantage of fluensulfone is its rapid action to kill nematodes. Exposure of one hour to fluensulfone had negative effects on *Caenorhabditis elegans*, after this time, 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* occurred post-fertilization [22]. 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]. This is a common action mode of nematicides, since other systemic nematicides have been reported to have inhibitory effects on egg hatching for various species of plant- parasitic nematodes [23-25].

The suggestion that fluensulfone inhibits feeding may be related to the fact that it induces larval arrest. Other chemicals have been shown to induce juvenile and embryonic arrest; however the mechanisms by which this arrest is induced are not known [26]. During larval arrest there is a known link between feeding and arrested development. Juveniles of nematodes can enter a diapause state when embryos hatch when food is scarce, during which they do not moult to the next stage until food becomes more abundant [26]. During larval arrest induced by nematicides, nematodes cannot feed, even in the presence of abundant food [25].

During *in vitro* experiments, fluensulfone has exerted detrimental effects on several plant-parasitic nematodes including *N. aberrans*, and *Meloidogyne arenaria,* the nematicide has prevented their increase to levels that could generate significant crop damage, thereby giving good protection to susceptible crops. Other works in the field have evidenced a significant decrease in populations of *M. arenaria* and *N. aberrans* by fluensulfone applications in

Table 1. Initial (Ip) and final (Fp) population densities of *Nacobbus aberrans* (J2·100 g^{-1}soil) 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= 0.05)

Treatment	Nematicide	Dosage ($L\cdot ha^{-1}$)	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}

tobacco and tomato. In these experiments fluensulfone was especially effective during pretransplant applications [7] that reduced the size of J2 inoculum in soil. This is one of the main principles underlying soil treatments with nematicides, given the direct relationship between the infection activities of the 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 low concentrations and short exposure periods [27]. Oxamyl 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 [6].

3.2 Percentage of Root Galling Index

Galling index values in cucumber roots 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). From these treatments the lowest GI was obtained at a dose of 2.25 L·ha^{-1}. The highest values were found in plants from control plots as was expected.

3.3 Efficacy of Nematicides against *Nacobbus aberrans*

There was no significant differences in the percentage of control of nematicides according to treatments, but the best control against *N*. *aberrans* was obtained in plots treated with fluensulfone at doses of 2.25 and 2 L·ha^{-1}. These treatments resulted in 81.15 and 80.33 percentage of control, respectively (Fig. 1). The results agreed with other studies that indicate an efficient control of *N. aberrans* by fluensulfone, which is explained in part to the irreversible nematicidal effect of the nematicide [6,28].

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

3.4 Growth Parameters

Vine length of cucumbers showed no statistical differences (P=0.05) according to nematicide treatments. Mean values for vine length ranged 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}. Fresh root weight showed no statistical differences (P= 0.05) among treatments, with average values ranging between 7.18 and 8.21 g per plant (Table 3). The greater weight of roots was obtained from the plots treated with oxamyl at a dose of 3 L·ha^{-1}.

Variable population densities of *N. aberrans* in soil and roots did not translate into differences in plant parameters of cucumber. Our results agreed with previous studies that report a positive effect of the application of fluensulfone and oxamyl on biomass production of different crops [6,28]. Additionally, this finding can be explained in part by the application of fertilizer that could partially, offset nematode-induced damage by stimulating plant development. Other studies have evidenced positive effects of macro and micronutrients on plant growth (i.e., cotton, sugarcane) in presence of plant-parasitic nematodes such as *Rotylenchulus reniformis* and *Meloidogyne* spp. [29-31]. It is likely that nitrogen in ammonium form that is present in the fertilizer applied to cucumber crop, had a negative effect on nematodes due to its plasmolytic effect around the point at which it is applied to the soil.

Table 2. Percentage of galled roots (PGR) and gall index (GI) observed in cucumber 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= 0.05)

Treatment	Treatment	Dosage (L·ha^{-1})	PRG	GI
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}

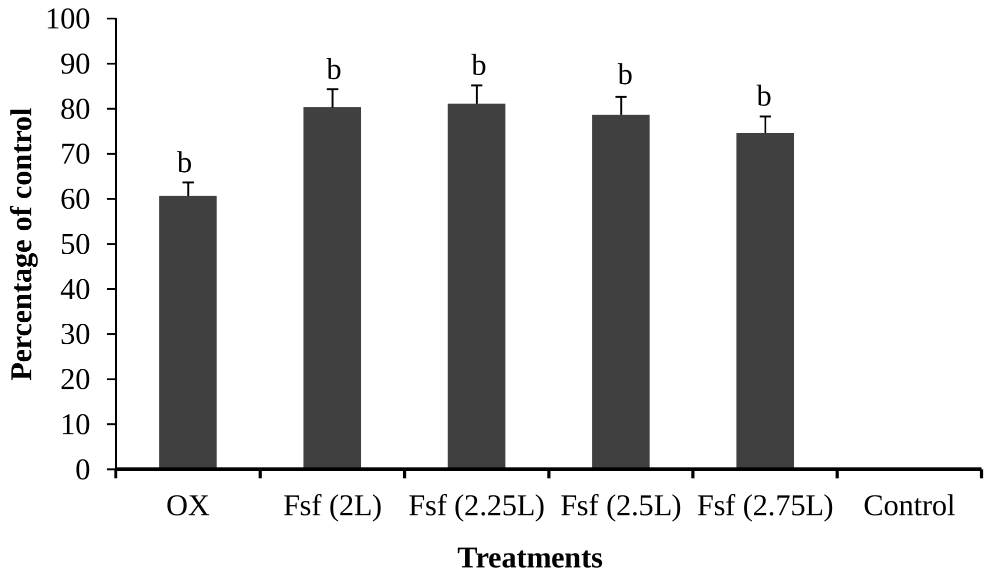

Fig. 1. Percentage of efficacy of the nematicide fluensulfone (Fsf) at different doses, and oxamyl (OX) during control of *N. aberrans* in cucumber plants. Means followed by the same letters on each bar are not significantly different (P= 0.05)

Table 3. Vine length (VL) and fresh root weight (FRW) observed in cucumber 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= 0.05)

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

It has been observed that plants with high levels of phosphorus, release fewer root exudates and therefore are less attractive to plant-parasitic nematodes, therefore decreasing the incidence of the diseases. Plants become more resistant when supplied with sufficient quantities of phosphorus as a result of increases in protein synthesis, cell activity and production of polyphenols, peroxidase and ammonia [31,32]. Other indirect mechanisms, such as an increase in microbiota antagonistic to nematodes, may also have a significant effect, many fungi in the soil prefer nitrogen in ammonium form (from fertilizer), which means that ammonia-producing materials could encourage proliferation of nematode parasitic fungi. Due to its nematicidal effect, urea has also been the subject of many studies, urea is rapidly converted into ammonia by the action of urease in the soil [31,32].

4. CONCLUSION

The present study revealed that application of fluensulfone under experimental conditions in cucumber crop provided a good control of the false root-knot nematode *N. aberrans*. The lowest nematode populations were obtained in plots treated with fluensulfone at dose of 2.25 $L^{.}ha^{-1}$. Fluensulfone can be suggested as an alternative nematicide for the control of *N. aberrans* on cucumber crop. Further evaluation of this nematicide should be carried out in different crops to 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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