

Effect of Vinasse in the Suppressiveness to *Pratylenchus brachyurus* in Soybean

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Abstract

Vinasse, a byproduct result of the process of distillation and fermentation of sugar cane in the ethanol production, stands out for high availability and nutritional alternative source in pest control. The objective of this study was to evaluate the vinasse potential applied to soil, aiming at the control of *Pratylenchus brachyurus* in soybean crop. The experimental design was a completely randomized factorial (2 × 11), consisting of two forms of application of vinasse (single and divided into two), in eleven concentrations (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), with five replications. The soybean plants were inoculated with a suspension of 4,000 eggs/juveniles of *P. brachyurus*. 60 days after the first application of vinasse, agronomic variables and parasitism were evaluated. The single vinasse application promoted greater growth and root development. Regarding the nematodes parasitism, the application divided was more efficient in the reduction of juveniles in the root and soil, where the concentrations of vinasse enough to reduce the population of 50% (CL₅₀) of the nematodes were 10.22% and 16.64%, respectively. Whereas, for the other variables such as: nematodes per gram of roots (73.97%), eggs in the root (86%) and total nematodes in roots and soil (67.90%), the greatest reduction was observed in the 20% concentration of vinasse. Therefore, vinasse shows potential as a nematicide, as well as excellent organic fertilizer.

Keywords: *Glycine max*, root lesion nematode, sugar and alcohol subproduct

1. Introduction

The soybean culture (*Glycine max* L.) is considered the most important oilseed in Brazil, which makes it the second largest producer in the world, with production exceeding 107 million tons to an area of 33 million hectare (CONAB 2017). However, various biotic factors, for example, Phyto nematodes, have been a limiting factor to crop production in all producer regions of the country (Inomoto et al., 2010).

The knowledge of the Phyto nematodes aggressiveness, by the high incidence and considerable losses in production, has led to the identification of the main species of greater occurrence, as the cyst nematode *Heterodera Ichinohe glycine* (1952), the root-knot nematodes *Meloidogyne javanica* (1885 Treub) Chitwood, 1949, *M. incognita* (Kofoid & White 1919) Chitwood 1949; root lesions *Pratylenchus brachyurus* (Godfrey 1929) Filipjev & Stekhoven 1941 and the reniform *Rotylenchulus reniforme* (Linford & Oliveira 1940), considered key nematodes, for the soybean crop (Ferreira, 2010).

Among the mentioned species, *P. brachyurus* deserves to be highlighted, of greater occurrence in the Cerrados of Brazil (Asmus, 2004). Considered a migratory pathogen endoparasite, which upon penetrating the roots by the mechanical action of its stylet, causes the roots decomposition, by means of enzymes and toxins, accelerating the plants cell walls degradation (Goulart, 2008).

The adaptation capacity of *P. brachyurus*, to the most diverse climatic conditions of the producing regions of Brazil, allied to the species polyphagia of the species, are limiting factors in the management measures adoption. However, among the main methods of control to Phyto nematodes, the defensive chemicals stand out, by

presenting a fast and easy handling result. However, their recommendation has been suffering restrictions around the world, due to their high toxicity to man and the environment, in addition to the high costs (Dong & Zhang, 2006).

Before the numerous negative effects that pesticides can promote, especially when used in compliance with the manufacturer's technical standards, is increasing the development of research in the area of less aggressive products to nature, and which are readily biodegradable. In this sense, the vinasse, liquid residue resulting from the process of sugar cane distillation and fermentation in the ethanol production, with a high concentration of nutrients, mainly potassium (K), and organic matter, in addition to a high rate of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which demonstrates a perspective of natural product with potential nematicide (Albuquerque, 2002), and allelopathic, on the weeds emergence (Voll, 2005). In addition, vinasse promotes improvement of the soil physical and chemical properties, due to the increased capacity to retain moisture, porosity, mineral and conductivity (Lyra et al., 2003; Silva & Cabeda, 2005).

Information about the use of vinasse for pathogenic agents control are quite scarce, although it is known that the vinasse increases the biological activity in the soil, which favors potential antagonistic microorganisms, as well as promotes the organic substances formation, such as volatile fatty acids, which may have nematicide action (Tenório et al., 2000). Therefore, the objective of this study was to evaluate the vinasse effect applied to the soil in the control of *P. brachyurus* in soybean crop.

2. Material and Methods

2.1 Location and Experiment Conduction

The experiment was carried out in greenhouse and in the Laboratory of Pathology, Federal University of Piauí, Campus Professor Cinobelina Elvas, on Bom Jesus, PI, in the period from april to august 2015.

The substrate used was a mixture of soil-sand-manure, in the ratio 3:2:1, previously sterilized in vertical autoclave, at 120 °C and 1.05 Kg cm² for two hours. Soon after, it was placed in plastic pots with capacity of 5.0 dm³ laid down on bench. After analysis, the substrate showed the following characteristics: Dystrophic red Latosol, medium-sandy texture; pH = 6.2 (H₂O); organic matter = 15.8 (g kg⁻¹); sand = 710 (g kg⁻¹); silt = 50 (g kg⁻¹); clay = 240 (g kg⁻¹); Ca⁺² = 2.6 (cmolc dm⁻³); Mg⁺² = 1.3 (mg dm⁻³); exchangeable P = 108 (mg dm⁻³); K⁺ = 88.0 (mg dm⁻³) and Zn = 6.7 (mg dm⁻³).

The inoculum was obtained from a population of *P. brachyurus* in soybean crop in the region of Bom Jesus-PI. The extraction of eggs and juveniles (J2), occurred by liquefaction and centrifugation in sucrose solution with kaolin, according to the method of Coolen and D'Herde (1972). After extraction, the inoculum was maintained in corn (*Zea mays*) hybrid Pioneer 30F53, grown in a greenhouse for multiplication. For the identification of this species, temporary slides were used (formalin) and/or permanent (glycerine), examined under a light microscope, confronting the morphological characteristics (format of structures) and morphometric (dimensions of structures and linear bodily relationships) with the use of taxonomic keys specific to the genus (Handoo & Golden, 1989).

The experimental design was a completely randomized factorial 2 × 11. The vinasse application was performed as follows: Single (application of 100 ml pot⁻¹) and divided into two (two applications of 50 ml with 15-day interval between them), in eleven concentrations (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%), with five replications, via soil, totaling one hundred and ten plots in the study.

Vinasse was obtained from small distilleries in the region of Palmeira-PI, in function of the availability in the craft cachaça production. The physico-chemical composition of the pure vinasse, employed in the assay, showed the following characteristics: N (0.36 kg/m³); P₂O₅ (0.13 kg/m³); K₂O (1.43 kg/m³); Ca (0.41 kg/m³); Mg (0.21 kg/m³); S (1.28 kg/m³); organic matter (18.49 kg/m³); Fe (53.15 ppm); Cu (5.84 ppm); Zn (1.07 ppm); Mn (3.21 ppm); pH in water (3.8); DBO (25.285,00 mg L⁻¹); DQO 38.785,00 mg L⁻¹) and CE (8.12 ds m). To maintain a better conservation, the product was kept in glass pots and placed in the refrigerator, 4 °C, until the application period.

The sowing was performed with five Intacta cv. soybean seeds. M-Soy 8644 IPRO per pot. After 7 days emerging, the thinning was carried out leaving two plants per pot, constituting the experimental unit. After 72 hours of thinning, a suspension of 4,000 eggs/juveniles of *P. brachyurus* was inoculated, with the aid of a pipette, adding 10 ml of inoculum solution along three holes (3.0 cm of depth), with spacing of 2.0 cm from the soybean plants hypocotyl.

At 10 days after inoculation, the vinasse was applied separately in each vessel, according to the respective application forms and concentrations employed. The concentrations were determined using vinasse dilutions in distilled water and prepared 2 hours before the soil treatments application.

During the experiment, the temperature (room and soil) and relative air humidity were monitored with temperature in the greenhouse in an average of 28-35 °C, with a soil temperature of 25-32.5 °C and a relative humidity of 25-50%.

2.2 The Evaluations Were Carried

The evaluations were performed at 60 days after the vinasse application. Corresponding the following agronomic variables: root length—using graduated ruler; fresh root mass—with the aid of semi-analytical scales; and roots volume—calculated by the difference between the volume of water without the roots (400 ml) and the volume after the roots being immersed, using a 1000-ml beaker. For the parasitism characteristics the nematodes population was evaluated from the root system, used for the assessment of population density, where juveniels in the root and eggs in the root were evaluated, using the methodology described by Coolen and D’Herde (1972). Regarding the soil nematode population, juveniles in the soil and eggs in the soil were evaluated, from soil samples with 300 cm³, using the flotation and centrifugation technique in sucrose solution (Jenkins, 1964). After the nematodes quantification, it was also calculated the reproduction factor (Rf) of the parasite for each treatment, using the method proposed by Oostenbrink (1966).

2.3. Statistical Analysis

The agronomic variables and parasitism data were subjected to analysis of normality by the Shapiro-Wilk test and analysis of variance (ANOVA) by F test ($p < 0.05$). When significant, the means for the forms of vinasse application were compared by the Tukey test ($p < 0.05$), using the statistical program “R” version 3.3.2. Whereas, the means for the vinasse concentrations were used in a regression analysis performed with the aid of the software SigmaPlot 11.0.

3. Results and Discussion

Through the analysis of variance it is observed that there was no significant interaction ($p > 0.05$) between forms of vinasse application and concentrations, for agronomic variables (Table 1). However, all variables were affected by the actions of individual factors. For the purpose of application forms, there was a significant difference ($p < 0.01$) in all agronomic variables, whereas, for the purpose of concentrations, there was a significant difference ($p < 0.05$) only for the root length.

Table 1. Summary of the analysis of variance (F test and mean squares) for agronomic characteristics of soybean plants inoculated with *P. brachyurus*, in function on the form of vinasse application and concentrations

Source/variation	Soybean agronomic trait		
	Root length	Root volume	Root fresh mass
Application form (AF)	2137.52**	2974.40**	1919.54 **
vinasse concentration (CCv)	171.28*	56.23ns	124.25ns
FA × CCv	48.60ns	58.54ns	93.24ns
CV (%)	17.21	25.04	22.81

Note. **: Significant at 1%; *: Significant at 5%; ns: not significant; CV (%): Coefficient of variation.

The growth and development of the soybean plants root system were affected by forms of vinasse application, with emphasis on single application, regardless of the concentration, provided the highest averages for the length (53.83 cm), volume (43.31 cm³) and fresh mass (44.15 g) of the root system (Table 1). Results of vinasse efficiency o as Biofertilizers and/or Biostimulants, have already been described in different cultures. Silva et al. (2014), highlight gain of increment of 7, 10 and 15 t ha⁻¹, respectively, for sugarcane 1st ratoon, 2nd ratoon and 3rd ratoon, after addition of vinasse. According to Basso et al. (2013), the vinasse can be used as a source of K in succession black oat/corn silage/out-of-season corn in replacing the mineral fertilization with potassium chloride and with residual for the subsequent crops. Besides its importance as a source of nitrogen, the vinasse has stimulating potential in the improvement of germination and soybea, beans and rice seeds vigor, at concentrations lower than 20% (Galli, 2011).

For the purpose of vinasse concentrations on the root length, regardless of the form of application, the means were adjusted to a quadratic polynomial regression model, from which it is possible to optimize the concentrations with a view to maximising the average of this variable (Figure 1). The best concentration with higher expression in the gain for the evaluated parameter was observed with 54.63% of vinasse, which resulted

in an increase of 24.58%. It is possible that this effect is directly related to the nutrients present in the vinasse, with a large concentration of biodegradable organic matter, high concentration of potassium sulphate, calcium, magnesium and nitrogen, which gives it a high potential as natural fertilizer in replacing the mineral fertilization (Oliveira et al., 2014).

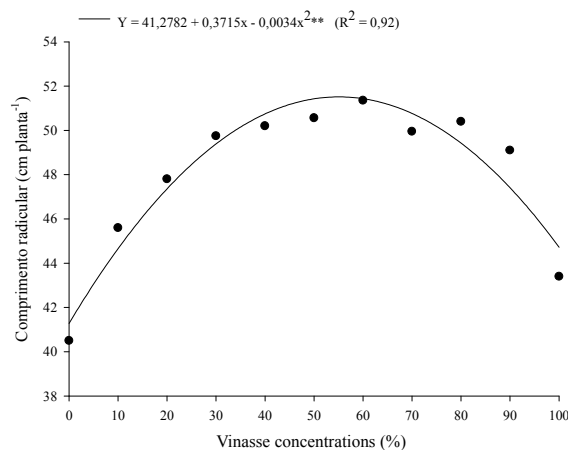


Figure 1. Root length of soybean plants in function of the vinasse concentrations

However, there is a decrease in the pronounced root growth of plants for concentrations above the ideal (Figure 1). These results express the care that must be taken upon using the vinasse without moderation, for example in the fertigation, particularly in view of the salinisation ability of this product, in addition to leaching of organic ions, such as potassium nitrate, and metals, which results in a short space of time, increase of phytotoxicity (Oren et al., 2004).

For the parasitism variables, there was interaction between the vinasse application forms and concentrations, with significant effect ($p < 0.01$) only for the number of eggs in the root (Table 2). However, with the exception of the number of eggs in the soil, the remaining variables were influenced by the performance of vinasse individual concentrations. Whereas, for the application forms, only the number of eggs in the soil and nematodes per gram of roots, were not affected by the individual factor action. Dias et al. (2000), state that the antagonistic effects promoted by the vinasse, has a direct relationship by the organic matter availability, which enables an increase of biodiversity and release of toxic compounds during the decomposition, which contribute to the reduction of the nematodes population.

Table 2. Summary of the analysis of variance (F test and mean squares) and mean values for the parasitism variables of *P. brachyurus* in soybean plants in function of vinasse application forms (FA) and concentrations (CC_v)

Variation source	Parasitism variable of <i>P. brachyurus</i>						
	JR	NGR	JS	TNRS	ER	ES	RF
(FA)	99661.10*	0.37 ^{ns}	2329600.58**	3676108.01**	3360.58*	301.12 ^{ns}	0.229**
Single applications	301.4 a	8.93 a	1341.71 a	1715.27 a	54.67 a	17.49 a	0.42 a
Two applications	241.2 b	8.81 a	1050.66 b	1349.66 b	43.62 b	14.18 a	0.33 b
(CC _v)	272587.04**	486.61**	4440697.88**	7847724.95**	31507.06**	2101.75 ^{ns}	0.490**
FA × CC _v	3614.76 ^{ns}	9.03 ^{ns}	78392.74 ^{ns}	92244.51 ^{ns}	1739.68**	2694.17 ^{ns}	0.005 ^{ns}
C.V. (%)	6.91	18.1	7.51	5.17	17.23	125.68	18.78

Note. * Means followed by the same lower case letters in a column do not differ significantly by the Tukey test. ** Significant at 1%; * Significant at 5%; ns not significant. C.V.: Coefficient of variation. JR: juveniles in the root; NGR: Nematodes per gram of roots; JS: Juveniles in the soil; TNRS: Total nematodes in the root and soil; ER: Eggs in the root; ES: Eggs in the soil; RF: Reproduction factor.

For the number of juveniles in the root of *P. brachyurus*, there was a significant difference between the vinasse application forms, with emphasis to the divided application, which resulted in an average of this variable in 19.97% lower than the results obtained with a single application (Table 2). It is also observed that the behavior of efficiency of the vinasse application plot, was observed for the other variables. Pedrosa et al. (2005), highlight that applications of high concentrations with the byproduct of sugarcane, may cause deleterious effect on plants. This phytotoxic effect, may be related due to the presence of aconitic acid (AA) present in grasses and the vinasse (Voll et al., 2005). Therefore, it is perceived that the fractionated applications reduce the damage on plants and keep the nematicide action for longer period.

The vinasse division was more efficient in reducing the number of juveniles of *P. brachyurus* in the soil, the total number of nematodes in the soil and root and the number of eggs in the root, with respective values of these variables in 21.69%; 21.31% and 20.21% lower than those obtained with a single application. When analyzing the reproduction factor (RF) of *P. brachyurus* in soybean plants, there was no significant difference between the vinasse application forms, with emphasis to the divided application which reduced the value of this variable in 21.43% compared to the single application (Table 2).

The maintenance of the vinasse efficiency when applied in plots, reinforces the concern not to exceed the capacity of ions retention of each soil, since this product has a great amount of mineral and organic elements, which can suffer leaching, especially of nitrate or potassium (Brito et al., 2009).

The means of number of juveniles in the root (Figure 2A), nematodes per gram of roots (Figure 2B), juveniles in the soil (Figure 2C) and total nematodes in roots and in the soil (Figure 2D), adjusted to the decreasing exponential regression model in function of the vinasse concentrations employed. For these variables, the lethal vinasse concentrations (LC_{50}) were estimated by regression equation corresponding to 10.22%, 7.23%, 16.64% and 12.88%, respectively, sufficient to cause the mortality of 50% of the nematodes. However, maximum reductions in these variables of 65.38%, 73.97%, 69.56% and 67.90% were observed with vinasse from the respective concentrations of 20%, 20%, 30 % and 30%, compared to the control. The presence of organic matter in the vinasse allows when there is decomposition, the release of toxic compounds such as ammonia, butyric acids and volatile fatty acids, which justifies its nematicide action (Oka, 2010).

In general, it is observed that the vinasse promotes the plants protection against such soil pathogens, however, there is disagreement regarding the probable nematodes action. Pedrosa et al. (2005), state that the vinasse action in the Phyto nematodes reduction, is related to the predators and parasites proliferation. Whereas, Ricci et al. (2004), add that with the increase of biodiversity in the soil, which promotes the toxic compounds release from the organic matter decomposition, the byproducts become nematicide potential, in addition to increasing availability of nutrients, reinforcing the plants resistance mechanisms to the most varied soil pathogens.

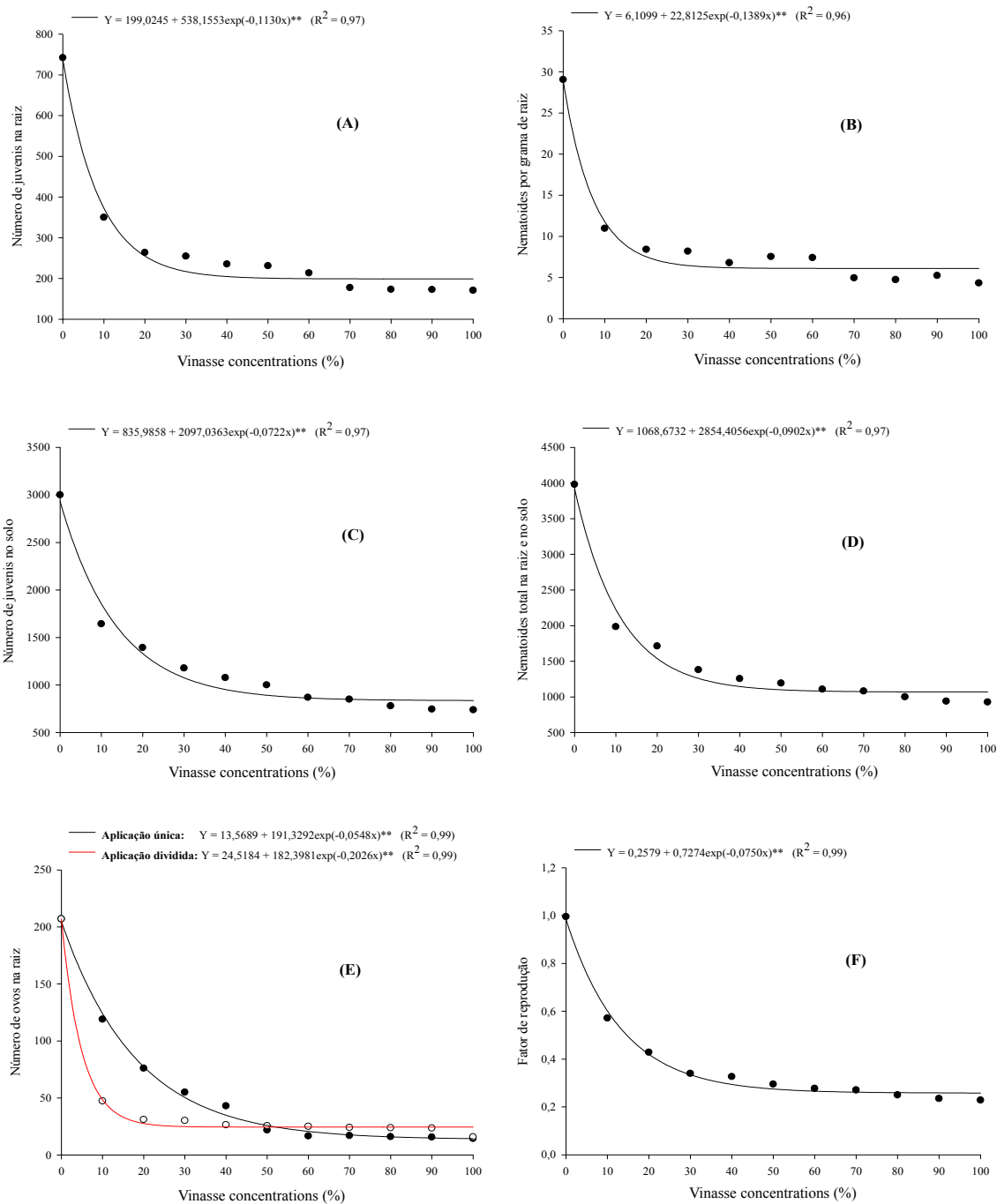


Figure 2. Number of juveniles in the root (A), nematodes per gram of roots (B), the number of juveniles in the soil (C) total nematode in roots and in the soil (D), the number of eggs in the root (E), and reproduction factor (F) of *P. brachyurus* in soybean plants, depending on the vinasse application forms and concentrations

The vinasse application forms influenced negatively the number of eggs of *P. brachyurus* in the root, with exponential decrease in the average of this variable in response to the concentrations employed (Figure 2E). The smallest means of this variable were also observed with divided application, because with only 4.13% of vinasse was possible to have a reduction of 50% of the eggs in the root, reaching a maximum percentage of reduction (86.62%) with 20% of vinasse. Whereas, for the single application, the reduction of 50% of the eggs of the root was only possible with the vinasse application at 13.99%, and for more expressive reduction (87.35%) of this variable, it was necessary the application of a higher concentration (50%). These results corroborate with Pedrosa et al. (2005), who studying vinasse doses to the soil, observed suppressive effect on nematodes, with

reduction of the pathogen directly proportional to the volume used. To some extent, the high content of organic matter present in the vinasse, promotes various synergetic reactions, which in turn contributes as a source of food for many organisms with characteristics that are antagonistic, that can inhibit the parasitic activity of the plants nematodes (Ferraz et al., 2010).

In function of the concentrations employed, it is observed that the FR value was reduced exponentially in 50% after application of 15.08% of vinasse. More expressive reduction in this variable was observed with concentration from 30%, which reduced the control FR from 0.99 to 0.33, with percentage of decrease of 66.04% (Figure 2F). As it is realized, the reduction of this variable can be understood as a strong indication of suppressive potential of the vinasse to nematodes, either promoted by the plants fertilization, or even, by increasing microbial activity antagonistic, favored by the vinasse addition.

The results obtained in this study, with vinasse, at concentrations and application forms, even presenting efficiency with reduction in all parameters of the nematode parasitism of soya, the necessity for new studies deserves to be highlighted for monitoring in open areas, especially when it comes to culture that receives large contributions of inputs. Moreover, the nutritional richness that the vinasse presents, has deserved highlighting for a long time in sugar cane fertigation. Thus, vinasse could become as a viable alternative, of nutritional and nematicide nature, thus contributing to a clean agriculture, eliminating the harmful pesticides effects and for a sustainable environment.

4. Conclusions

The vinasse applied to soil reduces the population of *Pratylenchus brachyurus* and promotes a better soybean vegetative development.

The divided application form of vinasse offers better efficiency in reducing *P. brachyurus*.

The concentrations from 20% of vinasse reduces the *P. brachyurus* parasitism in soybean.

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