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RESEARCH ARTICLE

Management of root knot nematode *Meloidogyne incognita* using black soldier fly frass in soybean [*Glycine max* (L.)]

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ABSTRACT

Glycine max (L.) is a leguminous crop used by humans and livestock; it is an alternative protein source that offers almost the same quality as animal protein. Currently, its productivity has been affected by *Meloidogyne incognita,* which threatens food quality and security in Sub-Saharan Africa by 25-70%. Research has been conducted to extract the knowledge and inputs that will be effective in solving nematode problems, as the available methods are inefficient, inaccessible, and expensive. This study aimed to use BSFF in managing *M. incognita* in Soybean cultivation. The study was conducted in Mwanza-Tanzania, where seven treatments following a CRD design were inoculated with 1000 J2 of *M. incognita* and replicated six times. Results showed that BSFF from *Hermetia illucens* has the potential to minimize the effect of *M. incognita* in soybean, where the number of galls was reduced from 104 in 0.0 kg BSFF/inoculated to 3 in 1.0 kg BSFF/inoculated, followed by 6.33, 23.67, 51.51 and 52.50 galls in 0.75 kg BSFF/inoculated, 0.5 kg BSFF/inoculated, 50mL chitosan /inoculated, and 0.25 kg BSFF/inoculated, respectively. Also, symptoms such as chlorosis were reduced from nine leaves in 0.0 kg BSFF/inoculated to zero in 1.0 kg BSFF. Therefore, the study found that BSFF is a sustainable way to minimize the effect of M. incognita and maximize the performance of soybean production, as it offers soil management benefits, and enhances social and economic welfare by reducing the use of harmful, expensive chemicals that interfere human health and economy.

Keywords: black soldier fly, frass, management, root-knot nematode, soybean, sustainable.

INTRODUCTION

Food insecurity is a growing concern in several African countries, including Kenya, Uganda, Tanzania, Zimbabwe, Zambia, Rwanda, Burundi, and DR-Congo. The main drivers of food insecurity in Africa are conflict, climate extremes, pests, economic downturns, and slowdowns (FAO, 2019). In response to this challenge, efforts have been made to introduce crops that can help address food insecurity (FAO, 2013). Soybean is a notable summer legume with high protein and oil content, making it a valuable source of nutrition for both humans and animals. It has the potential to alleviate food insecurity and malnutrition in Sub-Saharan Africa, given the concerns surrounding the rapid increase in the global population (FAO et al., 2019).

However, soybean production faces various challenges, including parasitization by Plant-Parasitic Nematodes (PPN) (Sikora et al., 2018). The expansion of soybean cultivation has increased the risk of nematode infestation and subsequently caused significant damage to soybean crops (Sikora et al., 2018). Current nematode management techniques, such as crop rotation, resistant varieties, and chemical methods, have limitations and may not be fully effective (Mbatyoti, et al., 2020). There is a need for alternative control measures that are both effective and environmentally friendly.

Nematodes are a major cause of pre-harvest losses in Sub-Saharan African countries, leading to significant yield reductions. This study focused on the use of Black Soldier Fly Frass (BSFF) as a biological control measure against Root Knot Nematodes (*Meloidogyne incognita*) in soybean production. The study aimed to evaluate the effectiveness of BSFF in controlling nematode infestation and assess the performance of soybean plants treated with different levels and concentrations of BSFF.

The current methods of nematode control involving broad-spectrum pesticides have drawbacks, including their toxicity and long-lasting effects on the environment and soil micro-biota (Mbatyoti, et al., 2020). Therefore, the use of BSFF, which is a natural and ecologically sound input, offers a potential alternative for managing Root Knot Nematodes in soybean production. By testing the efficacy of BSFF under controlled conditions, this study seeks to provide insights into its potential as a sustainable and effective solution for nematode control in soybean crops.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Ministry of Agriculture Training Institute (MATI-Ukiriguru) Mwanza Tanzania, located 28 km south of Mwanza at 2° 43' 0" South, 33° 1' 0" East at 1236 m above sea level. MATI-Ukiriguru is the Public Institute bordering the Tanzania Agricultural Research Institute (TARI-Ukiriguru), which researches crops like Root and tubers, Beans, Cassava, and Cotton as main crops. The MATI Ukiriguru is mandated to coordinate the training and conduct minor researches with students and famers all around the zone. The place receives a Minimum Rainfall of 299.16 mm and approximately 14 rains per month where the temperature ranges from 25 ˚C during day and 19 ℃ during the night and Relative humidity of 74% (Abubakar et al., 2023). The Laboratory works were done at the Sokoine University of Agriculture in the Morogoro Region

Research Design

Experimental Materials

This study used Uyole Soya 2 variety seeds, Black Soldier Flies Frass (BSFF), Growing medium (3 Forest Soil:1 Rice husks), Planting Containers, irrigation water, Notebook, Pen, Irrigation sprayer, Plates, Two Scissors, Two buckets, Electronic Weigh Balance, Ruler. The Black Soldier Fly Frass was collected from the Black Soldier Fly farm found in Kibamba-Dar Es Salaam. This experiment was set in the screen house (greenhouse) to exclude some factors like pathogens, pests, and rain.

Experimental material preparation

The Frass was dried overnight in the oven at 60 °C prior to be used for hygienisation purposes. The collected Forest soil was mixed with rice husks to make a medium of 3: 1 ratio, then the medium was sterilized by using specialized local heat vessel to make sure all disease pathogens and weed seeds are avoided in the experiment. After sterilizing both BSFF and Medium then were measured at different amounts to make different Treatments; Treatment 1 (T1), Treatment 2 (T2), Treatment 3 (T3), Treatment 4 (T4), Treatment 5 (T5), Treatment 6 (T6) and Treatment 7 (T7). Therefore, this experiment used seven treatments with different amounts of BSFF, and replicated six (6) in assessing the effectiveness of black soldier fly frass in controlling nematodes (*Meloidogyne incognita*) in soybean production.

Experimental procedures

An inoculum of *M. incognita* at a juvenile stage (j2) was obtained from the infected tomato roots collected in different farms through an extraction method called tray extraction method/techniques. The tray method is also known as the Modified Baermann technique (pie-pan method or the Whitehead tray method), during this procedure the rinsed root samples were chopped into very small pieces and then placed onto the sieves for extraction, little water was added and left for 2 days while maintaining the wetness in the extraction container. After two days water was collected from the extraction and ready for nematode counting and inoculation (Coyne et al., 2007). Nematodes (*M. incognita*) at the J2 stage were counted in the laboratory and inoculated with a concentration of *1000 J2* per 5 mL solution. The CRD experimental design was followed to arrange the Treatments (T1: Zero Nematodes and Zero BSFF); (T2: *1000J2* Nematodes and Zero BSFF); (T3: 1000J2 Nematodes and 0.25kg of BSFF); (T4: 1000J2 Nematodes and 0.5kg of BSFF); (T5: 1000J2 Nematodes and 0.75kg of BSFF); (T6: 1000J2 Nematodes and 1.0kg of BSF Frass); (T7: 1000J2 Nematodes and Chitosan). The experiment was conducted in greenhouse conditions with six replications of each treatment. Inoculation of 1000 J2 stage *M. incognita* to soybeans was done two weeks after germination (Coyne et al., 2007).

Experimental layout

The pot experiment study was conducted using Completely Randomized Design (CRD) under Screen-house conditions. A total of 42 pots were used per experiment with one plant per pot. Inoculation of uniform stage and concentration of *M. incognita*-1000J2 in 5 mL was done two weeks after germination.

Methods of Data Collection

Above ground symptoms

The symptoms caused by *M. incognita* in the aerial part of the plant (leaves and shoot) include Chlorosis (yellowing), wilting, stunting, reduced number of pods and smaller pods were observed Coyne et al., 2007).

Underground symptoms

The plants were uprooted after maturity to check for RKNs occurrence, abundance, and severity of the pathogen by identifying the presence of root galls (Lima et al., 2017). The number and size of the galls indicated the degree of infection of root knot nematodes (*M. incognita*) (Elhady et al., 2018). The number of galls as an indication of root-knot nematodes in each treatment was recorded and rated from 0 to 5. 0 - No galls/plant; 1 – 1 to 2 galls; 2 – 3 to 10 galls/plant; 3 – 11 to 30 galls/plant; 4 – 31-100 galls/plant; 5 – 100+ galls/plant in roots as was discussed by Cenis 1993 and Niu et al., 2011.

Number of nematode galls per 1 gram of the root sample

The degree of nematode infestation after treatment application was assessed by counting the number of galls per 1 gram of root sample. Several small portion of a root (1 gram) was taken and the number of galls was identified and counted. The average number of galls per 1 gram root sample from the plant was recorded (Cenis, 1993 & Niu et al., 2011).

Number of pods per plant

The pods from each plant in each treatment of the nematode control experiment were counted and recorded. To determine the effect of black soldier fly frass on soybean yield, the number of pods per treatment was counted and compared (Keller et al., 2001).

Weight (g) of pods per plant

Plant pods were harvested by de-attaching the pods from the plants in all treatments. The pods were weighed by using a sensitive weight balance to determine their weight in grams. The weight was recorded from all treatments in all replications (Keller et al., 2001).

Soybean plant biomass

Plant biomass was determined before and after harvesting the pods from the plant. The whole plant (root, shoots, and pods) was uprooted, soil removed from roots, and weighed to determine its weight in grams. Also, plant biomass was determined by taking above-ground parts separated from below-ground parts of the plant to make above-ground and below-ground biomass respectively (Poorter et al., 2011).

Data Analysis

The normality of data and homogeneity of variance in effects of BSFF on Root Knot Nematodes control were checked using shapiro-wilk test and levene's test respectively. One-way analysis of variance (ANOVA) and two sample t-tests were used to analyze the data. The mean and standard error of each parameter were calculated and the separation of mean values between the treatments at $P \leq 0.05$ was done using post-hoc Tukey HSD. All the analyses were done using R-statistical software version 4.2.1.

RESULTS AND DISCUSSION

Effect of BSFF on leaves symptoms

Chlorotic plants and their rating were significantly ($P \leq 0.05$) different at different levels of treatments in soybeans ($F=21.44$, $DF=6$, $P=0.001$) (Table 1). There was averagely a higher number of chlorotic leaves in the treatment with 0kg BSFF (9.8 leaves) than in 0.25kg BSFF (5.67), 0.5kg BSFF (2.17 leaves), 50 ml chitosan (0.33leaves), 0.75 kg BSFF (0.17 leaves), control and 1kg BSFF which had no chlorotic leaves. The BSF frass has shown the ability to reduce the number of chlorotic leaves from 9.8 leaves in 0kg BSFF to zero leaves in 1kg of BSFF (Figure 1).

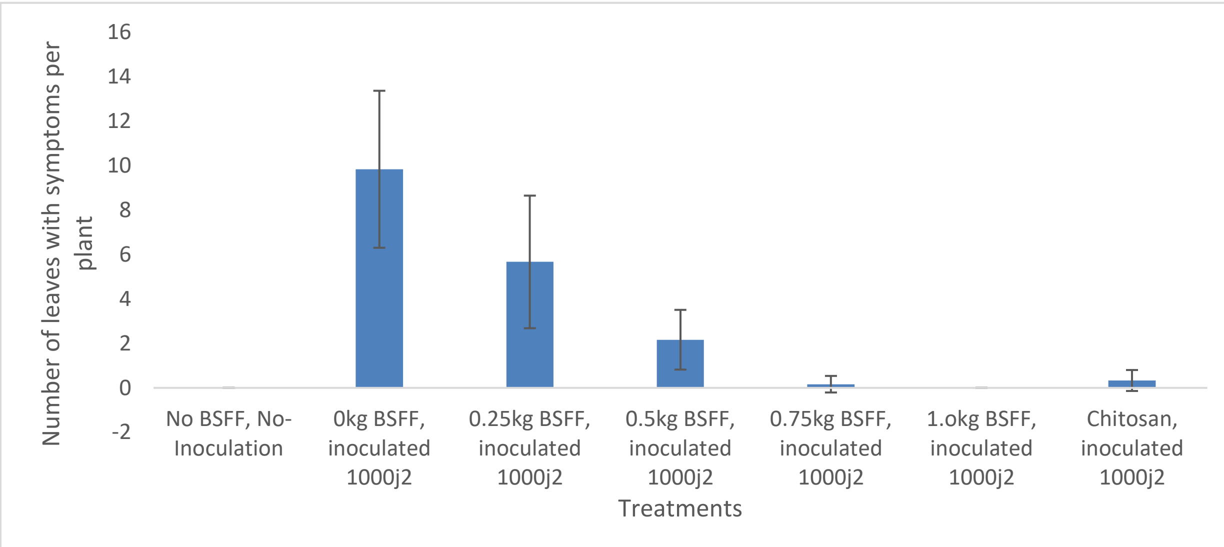

Figure 1. The effect of Black Soldier fly frass on the reduction of aerial symptoms in soybeans during root knot nematodes control

Effect of BSFF on Number of flowers

There was a significant ($P \leq 0.05$) difference observed in the number of flowers that appeared on soya beans planted with the seven treatments ($F=8.81$, $DF=6$, $P=0.001$) (Table 1). An average of 25.83 flowers were present in the treatment with 1kg BSFF,18.33 in 0.75kg BSFF, 12.5 in control, 12.33 in 50ml chitosan, 9.83 in 0.25kg BSFF,9.67 in 0.5kg BSFF and 4.50 in 0kg BSFF.

Effect of BSFF on the number of pods produced

The number of pods produced by soybeans was significantly ($P \leq 0.05$) different in the seven treatments ($F=32.42$, $DF=6$, $P=0.001$) (Table 1). The treatment with 1kg BSFF produced 76.67 pods followed by 0.75kg BSFF that produced 51.83 pods, Control at 37.5 pods, 0.25 kg BSFF at 35.83 pods, 0.5kg BSFF at 35.67pods, 50ml chitosan at 31.83 pods and 0kg BSFF at 22.33 pods (Figure 2).

Effect of BSFF on the weight of pods

The weight of pods produced by soya beans was significantly ($P \leq 0.05$) different at different treatments in soybeans ($F=55.66$, $DF=6$, $P=0.001$) (Table 1). The pods from 1kg BSFF had an average of 66.83g, followed by 0.75kg BSFF at 53.67g, 0.5kg BSFF at 41.55g, control at 37.67g, 0.25 kg BSFF at 34.50g, 50 ml chitosan at 25.83g and 0kg BSFF at 18.5g (Figure 2).

Effect on plant biomass weight

There was a significant ($P \leq 0.05$) difference in the plant biomass of soybeans planted observed in all seven treatments ($F=44.95$, $DF=6$, $P=0.001$) (Table 1). A significantly higher average amount of plant biomass was recorded in soybeans planted with 1kg BSFF (168.17g), followed by 0.75kg BSFF (160.17g), control (121.83g), 5kg BSFF (117.33g), 0.25kg BSFF (116.50g), 50ml chitosan (99.67g), and 0kg BSFF (85.50g).

Effect of BSFF on the number of galls per plant

The number of galls recorded from each soya bean that was planted in seven treatment conditions was significantly ($P \leq 0.05$) different ($F=49.98$, $DF=6$, $P=0.001$) (Table 1). There were no galls recorded in the control treatment. However, the number of galls from highest to lowest were recorded as 104.33, 52.50, 51.50, 23.67, 6.33, 3.00 and 0.00 in 0kg BSFF, 0.25kg BSFF, 50mls chitosan, 0.5kg BSFF,0.75kg BSFF, 1kg BSFF, and control, respectively (Figure 3).

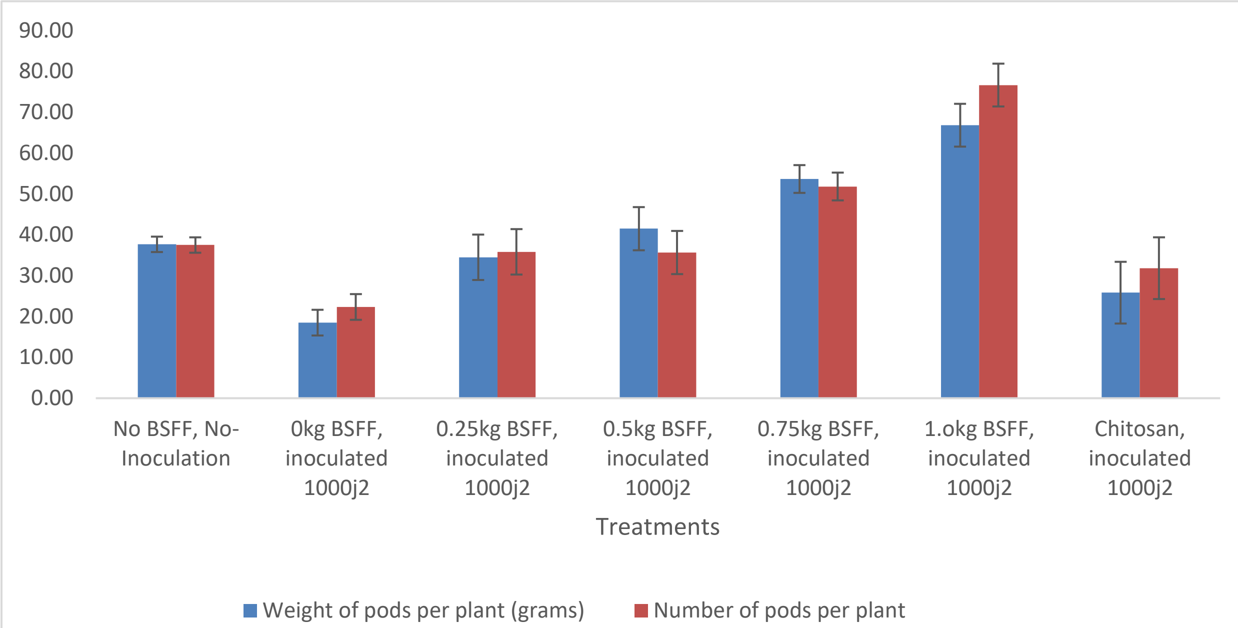

Figure 2. The effect of Black soldier fly frass and Chitosan on the number and weight of pods in soybeans during Root Knot Nematodes control

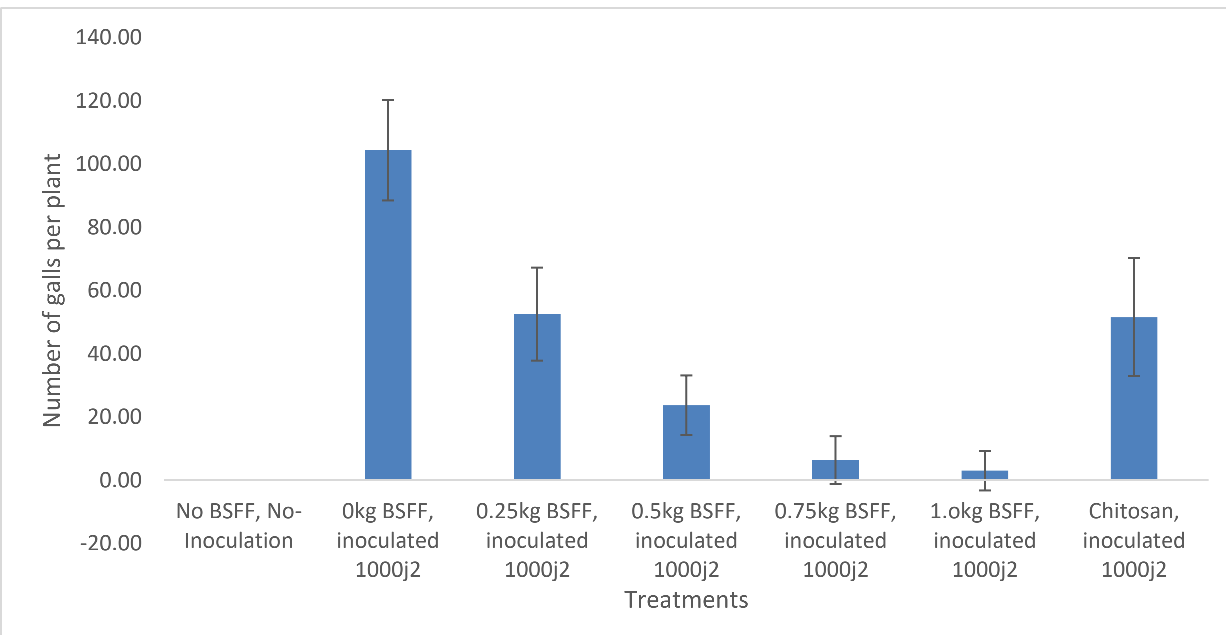

Figure 3. Effect of Black soldier fly frass on the number of galls (infestation) per plant in soybean during Root knot nematodes control

Effect of BSFF on the number of galls per 1g of root sample

Significant differences were observed in the number of galls present per 1g of root sample ($P \leq 0.05$) in all seven treatments ($F=9.76$, $DF=6$, $P=0.001$) (Table 1).

A higher number of galls per 1 gram of soil sample was recorded in the treatment with 0kg BSFF (9.33), followed by 50ml chitosan (5.33), 0.25kg BSFF (4.33), 0.5kg BSFF (3.17), 1kg BSFF (0.33), and control (0.00) (Figure 4).

Table 1. Aerial performance of soybean grown under different treatments of Black soldier fly frass and Chitosan in Root Knot nematodes control.

Parameters	Treatments							p-value
	Control (0kg BSFF, No nematodes)	0 kg BSFF and 1000j2 (nematodes)	0.25kg BSFF and 1000j2 (nematodes)	0.5kg BSFF and 1000j2 (Nematodes)	0.75kg BSFF and 1000j2 (nematodes)	1.0kg BSFF and 1000j2 (nematodes)	chitosan and 1000j2 (nematodes)	
Number of chlorotic leaves	$0.00±0.0^{a}$	$9.83±1.6^{c}$	$5.67±1.3^{b}$	$2.17±0.6b^{c}$	$0.17±0.2^{c}$	$0.00±0.0^{c}$	$0.33±0.2^{c}$	0.001
Number of galls per plant	$0.00±0.0^{a}$	$104.33±7.1^{cd}$	$52.50±6.6^{b}$	$23.67±4.2^{b}$	$6.33±3.4^{c}$	$3.00±2.8^{cd}$	$40.17±4.8^{d}$	0.001
Number of galls per 1g root sample	$0.00±0.0^{a}$	$7.00±1.2^{c}$	$3.50±0.5^{b}$	$2.00±0.3^{b}$	$0.33±0.2^{bc}$	$0.00±0.0^{c}$	$3.83±0.3^{c}$	0.001
Plant biomass(g)	$121.83±4.9^{a}$	$85.50±3.9^{bc}$	$116.50±4.6^{a}$	$117.33±7.7^{b}$	$160.17±2.4^{bc}$	$168.17±2.7^{cd}$	$99.67±2.8^{d}$	0.001
Number of flowers per plant	$12.50±1.2^{a}$	$4.50±1.2^{bc}$	$9.83±2.1^{ab}$	$9.67±1.9^{bc}$	$18.33±2.7^{bc}$	$25.83±4.2^{bc}$	$12.33±1.5^{c}$	0.001
Number of pods	$37.50±1.2^{a}$	$22.33±3.6^{cd}$	$35.83±2.2^{b}$	$35.67±1.7^{c}$	$51.83±1.9^{cd}$	$76.67±5.9^{cd}$	$31.83±2.9^{d}$	0.001
Weight of pods(g)	$37.67±0.9^{a}$	$18.50±1.4^{cd}$	$34.50±2.5^{b}$	$41.50±2.4^{c}$	$53.67±1.5^{c}$	$66.83±2.34^{de}$	$25.83±3.4^{e}$	0.001

Mean (± SE) percentage values with different superscript letters in the same rows are significantly different at P≤0.05

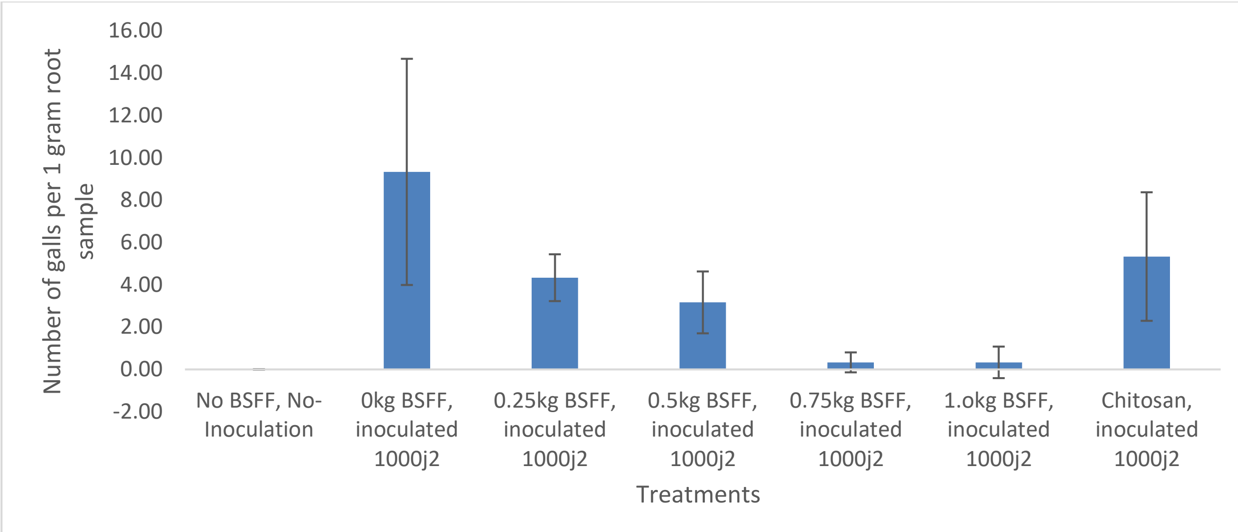

Figure 4. Effect of Black soldier fly frass and chitosan on number of knots (galls) per 1 gram of root sample in soybean during root knot nematode control.

DISCUSSION

This study has provided a general view of the potential advantage of Black Soldier fly (*Hermetia illucens)* and its frass in sustainable agriculture to control pathogens and contribute to crop productivity (López-López et al., 2010; Rodríguez-Navarro et al., 2011; Pagano & Miransari, 2016). The study focused on the utilization of BSFF and test its effectiveness and efficiency in different aspects of crop management. According to researches, BSFF is proved to compose chitin, microbial biomass, nutrients (NPK), growth hormone, soil enzymes, pesticidal, and antimicrobial properties (Saadoun et al., 2020). Chitin in the BSFF has been reported by Sharp (2013) to have several important beneficial effects on plant growth and health functions such as nematicide and fungicide.

BSFF from *H. illucens* used in this study, had a significant effect on root-knot nematode control in soybean production (Taylor & Sasser, 1978; Rich et al., 2009). As the amount of BSFF increased, the control of root-knot nematodes in soybeans improved. The results showed that the number of chlorotic leaves and root galls, which are major symptoms of nematode infestation on soybeans, decreased with increasing amounts of BSFF (Triantaphyllou, 1962; Rhoades, 1982; Rodríguez-Navarro et al., 2011). Treatment 6, with the highest amount of BSFF (1.0 kg), had the lowest number of chlorotic leaves and root galls, indicating effective nematode control. Since 1980's, researchers discovered chitosan as an essential soil improvement agent to control variety of soil borne parasites includes nematodes. According to Spiegel et al. (1988), clandosan (0.3% w/w) made from crustacean chitin was reported to reduce the number of eggs per cyst of *H. schachtii* by 44%.

Moreover, a similar experiment was done on the use of biopesticides from Essential oil against root-knot nematodes in Portugal, this included the oils from aromatic and medicinal plants, where geraniol showed promising antinematicidal activity against the pest (Faria, et al., 2022). Ijan and Mmbaga (2008), reported the ability of marigold plants to control nematode compared to ethylene dibromide which had phytotoxic effect on plants in the same experiment. The data presented in this work, suggest that the use of BSFF in soybean production can be an alternative measure for *M. incognita* control, since it is sufficiently available at low or no cost, especially considering the fact insect rearing like black soldier flies is currently of concern in Africa.

Furthermore, the study reveals the contribution of BSFF to soybean yield (Burton & Barker, 1998; Williamson & Roberts, 2009). The results showed that BSFF had a significant impact on various parameters related to soybean yield, including the number of flowers per plant, number of pods per plant, weight of pods per plant, plant biomass, shoot and root length during root knot nematodes control (Holshauser & Eisenback, 2011). The highest performance in terms of the number of pods and their weight per plant was observed in treatment 6, which received 1.0 kg of BSFF. As it was reported by Faria, *et al*. (2022) and Ijan and Mmbaga (2008), most of currently existing bio-pesticides only serve pest control function and do not help increasing yield productivity of the plant. Data obtained in this study has shown the ability of the BSFF to control the parasite while guaranteeing the performance of the plant. However, the study did not determine the optimal concentration of BSFF for *M. incognita* control for soybean production improvement.

This study has demonstrated the potential benefits of using insects in sustainable management of plant pathogens and enhance crop performance (Gamliel & Stapleton, 1993; Everts et al., 2007; Oka, 2010). The results contribute to the biological control measure against Root Knot Nematodes in soybean production. Further research is needed to determine the optimal concentration of BSFF and explore other insect frasses for similar applications

CONCLUSION

This study has provided valuable insights into the potential advantages of Black Soldier Fly Frass (BSFF) in sustainable agriculture for pathogen control, specifically in the case of Root Knot Nematodes (*Meloidogyne incognita*), and its contribution to enhancing soybean crop productivity. These findings highlight the potential of BSFF as a biological control measure for nematode management in soybean production, with the added benefit of enhancing crop yield. Additionally, the study emphasizes the importance of further research to determine the optimal concentration of BSFF and explore the use of other insect frass in similar applications.

Based on the results obtained from this study, the following recommendations are proposed: Further Research: Conduct further research to determine the specific concentration of BSFF and Chitosan that can minimize the nematode effect on soybeans. This will provide more precise guidelines for farmers and practitioners in implementing BSFF as a sustainable and effective solution. Expand Crop Studies: Extend the investigation to other crops affected by nematode infestation to assess the effectiveness of BSFF and Chitosan in diverse agricultural systems. This will provide a broader understanding of the potential benefits and limitations of these biological control measures. Sustainability Assessments: Conduct comprehensive assessments to evaluate the long-term sustainability of BSFF application, considering factors such as ecological impact, economic viability, and social acceptance. This will help ensure that the implementation of BSFF aligns with sustainable agricultural practices. Farmer Education and Adoption: Develop educational programs and initiatives to raise awareness among farmers about the benefits of using BSFF as a biological control measure. Provide training and technical support to facilitate the adoption of BSFF and promote its integration into sustainable crop management practices. Policy Support: Encourage policy support and incentives for the adoption of BSFF and other biological control measures in agriculture. Foster collaborations between researchers, policymakers, and industry stakeholders to facilitate the development and implementation of sustainable agricultural practices

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CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Andrea Malima Kigeso performed the experiment, collected, analyzed, interpreted the analyzed data as well as wrote the manuscript. Darius O. Andika make all the editing and review. Yasinta B. Nzogela designed the experiment.

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