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RESEARCH ARTICLE

Application of *Ascophyllum nodosum* extract and its nutrient components for the management of *Meloidogyne javanica* in soybean

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ABSTRACT

Nematodes of the *Meloidogyne* genus are among the main limiting factors of world agricultural productivity. Its management is quite complex and alternative methods such as the use of extracts have been widely studied. This study aimed to evaluate the penetration and development of *M. javanica* in soybean (*Glycine max* (L.) Merr.) treated with *Ascophyllum nodosum* extract and assess this algal product and its nutrient components in nematode management. Experiment 1 comprised five sampling times (5, 10, 15, 20, and 25 d after nematode inoculation) and three application methods (planting hole, foliar spraying, and untreated control). Soybean plants were inoculated with 2000 eggs+second-stage juveniles of *M. javanica* and treated with algal extract. Total nematode number, number of nematodes per developmental stage, and population density (nematodes g^{-1} root) were determined. Experiment 2 consisted of 11 treatments: Algal extract, N, K, P, Ca, Mg, Cu, Zn, Mn, Fe, combined nutrients, and untreated control. Soybean crops were evaluated for total nematode number, population density, gall index, plant height, root fresh weight, shoot fresh weight, and shoot dry weight. In Experiment 1, algal extract reduced nematode penetration, regardless of application method. There was a decrease in the total number of nematodes and number of nematode g^{-1} root by 83% and 56% in the application via furrow and 68% and 70% via foliar application, respectively. In Experiment 2, all nutrient treatments reduced at least one nematode parameter, with P, Ca, Mg, Fe, and combined nutrients (Trial 1) and algal extract, P, Ca, Mg, Cu, Zn, Mn, Fe and combined nutrients (Trial 2) achieving 83% to 95% reductions in population density. Nutrient treatments positively influenced vegetative development, as a 20% increase in height for the nutrients *A. nodosum*, N, K, P, Ca, Mg, Cu and Fe. *Ascophyllum nodosum* and its nutrient components have potential in the management of *M. javanica* in soybean.

Key words: Alternative control, *Glycine max*, nutrition, root-knot nematode, seaweed.

INTRODUCTION

Marine algae have been used for decades as fertilizers and soil conditioners and are believed to exert plant-stimulating effects by increasing resistance to abiotic and biotic stresses (Nanda et al., 2022). Alginates present in algae can act as elicitors of plant defense responses to pathogens (Ali et al., 2019). Research has shown that biostimulants based on marine algae have the potential to induce plant resistance and contribute to the control of some diseases (Rinaldi et al., 2021). Studies demonstrated the ability of marine algae to reduce the number of *Meloidogyne incognita* in cucumber (El-Eslamboly et al., 2019) and the number of *M. chitwoodi* Golden, O'Bannon, Santo, & Finley and *M. hapla* Chitwood eggs in tomato (Ngala et al., 2016).

Ascophyllum nodosum (L.) Le Jolis is one of the most studied algae for use as raw material in industrial and agricultural applications. This brown alga contains high levels of phenolic compounds, such as phlorotannins (oligomers or polymers of phloroglucinol), which possess antimicrobial action, in addition to polyphenols, betaines, polysaccharides, fatty acids, steroids, polyamines, plant hormone analogs, and macro- and micronutrients (Pereira et al., 2020). *Ascophyllum nodosum* has been described as one of the most efficient algae for the control of plant-parasitic nematodes (Williams et al., 2021). A study reported that *A. nodosum* was able to successfully control *M. javanica* in soybean crops via resistance induction through activation of enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and 1,3-glucanase (Rinaldi et al., 2021).

Another factor that aids in the defense of plants against pathogens is adequate nutrition, explained by the key role of nutrients in several metabolic functions (Mattos Jr. et al., 2017). Nutrients can have beneficial effects on some nematode-plant interactions, promoting the accumulation of cellulose, lignin, and other elements that confer resistance against infection (Santana-Gomes et al., 2013). Nutrients also participate in the synthesis of enzymes responsible for plant defense mechanisms (Couto et al., 2016).

Algae are rich in macronutrients such as N, K, P, Ca, and Mg and micronutrients such as Cu, Zn, Mn, Fe, B, Co, and Mo (El-Shenody et al., 2019). According to Van Oosten et al. (2017), cold stress induced by nutrient deficiency can be ameliorated by application of marine algal extracts rich in micronutrients, attributed to enhanced tolerance to oxidative stress. *Ascophyllum nodosum* extracts were shown to stimulate crop growth and yield by increasing nutrient absorption and availability (Van Oosten et al., 2017). Likewise, two commercial extracts of *A. nodosum* increased the macronutrient (N, P, K, Ca, and S) and micronutrient (Mg, Zn, Mn, and Fe) contents of tomato fruits (Di Stasio et al., 2018).

Based on the above observations, it was hypothesized that application of *A. nodosum* extract to soybean crops may reduce *M. javanica* reproduction and stimulate plant development through the action of nutrients and other compounds that enhance nematode resistance. This study aimed to evaluate *M. javanica* penetration and development in soybean treated with *A. nodosum* extract and assess the potential of the algal extract and its nutrient components in nematode management.

MATERIAL AND METHODS

General experimental procedures

Experiments were conducted in a greenhouse (23°24′17″ S, 51°56′26″ W; 313 m a.s.l.) and in a laboratory (23°24′18″ S, 51°56′29″ W; 313 m a.s.l.) For greenhouse experiments, the experimental units consisted of polystyrene cups containing 0.5 L 2:1 mixture of soil (Red Latosol, Type 3) and sand planted with soybean (*Glycine max* (L.) Merr.) ‘M6210 IPRO’. The substrate was autoclaved (120 °C, 2 h) before use.

Table 1. Nutrient composition of a commercial product based on *Ascophyllum nodosum* and equivalent products used in the study. [1]Composition analyzed by Agrisolum Laboratory, Maringá, PR, Brazil. Laboratory results differed from the nutrient composition declared by the manufacturer in the product datasheet (2% w/w N, 15% w/w K, and 25% w/w total organic C). [2]All reagents were purchased from Dinâmica Química Contemporânea Ltd., Indaiatuba, SP, Brazil.

Nutrient	Analyzed concentration[1]	Source	Equivalent product[2]	Rate (g L^{-1})
N	10.42 g kg^{-1}	CH_4N_2O	Urea P.A. ACS	1.62
K	142.44 g kg^{-1}	K_2SO_4	Potassium sulfate P.A. ACS	23.80
P	0.66 g kg^{-1}	P_2O_5	Phosphorus pentoxide P.A. ACS	0.18
Ca	3.95 g kg^{-1}	CaO	Calcium oxide P.A.	0.41
Mg	2.11 g kg^{-1}	MgO	Magnesium oxide P.A. ACS	0.26
Cu	20.67 mg kg^{-1}	$CuSO_4 \cdot 7H_2O$	Copper(II) sulfate ICO P.A. ACS	0.006
Zn	37.47 mg kg^{-1}	$ZnSO_4 \cdot 7H_2O$	Zinc sulfate P.A. ACS	0.012
Mn	28.42 mg kg^{-1}	$MnSO_4 \cdot H_2O$	Manganese(II) sulfate (OSO) P.A. ACS	0.0065
Fe	3591.76 mg kg^{-1}	$Fe_2(SO_4)_3 \times H_2O$	Iron(III) sulfate ICO P.A.	0.960

Soybean plants were treated with a commercial product based on an aqueous extract of *Ascophyllum nodosum* (Ativa Power, Alternativa Agrícola, Mogi Guacu, SP, Brazil), whose analyzed nutrient composition is presented in Table 1. The product was diluted in water and applied at a rate of 7.5 kg ha^{-1} and a spray volume of 100 L ha^{-1}, equivalent to 75 g L^{-1}. The rate was determined based on the results of a previous study assessing the control of *Meloidogyne javanica* (Rinaldi et al., 2021). After preparation, the extracts were transferred to flasks, wrapped with aluminum foil, and placed on a shaker for 24 h. After this period, the material was filtered through sterile gauze for use in the experiments.

The nematode inoculum was obtained from a pure population of *M. javanica* maintained on tomato (*Solanum lycopersicum* L.) 'Santa Clara' under greenhouse conditions. For extraction of eggs and second-stage juveniles (J2), tomato roots were processed according to the method of Boneti and Ferraz (1981). After extraction, nematodes were counted in a Peters' chamber under an optical microscope, and the suspension was calibrated to 2000 eggs+J2 mL^{-1}.

Experiment I. Penetration and development of *M. javanica* in soybean treated with *A. nodosum* extract

The first experiment was conducted in September 2020, with mean minimum, maximum, and average temperatures of 21.7, 23.9, and 22.8 °C, respectively. Treatments followed a completely randomized design, with a 5 × 3 factorial arrangement and five replicates. The factors were sampling time (5, 10, 15, 20, and 25 d after nematode inoculation, DAI) and method of extract application (planting hole, foliar spraying, and untreated control). Distilled water was used as a control. For in-hole application, a 4 cm deep hole was made in the soil of each pot and the extract was applied by sprinkling. Then, one seedling of soybean at the V2 stage was transplanted into the hole. Subsequently, pots were inoculated with 2000 eggs+J2 of *M. javanica* into two equidistant holes around the seedling. Foliar treatments were applied after transplanting and nematode inoculation. Leaves were sprayed to the point of runoff while the soil was protected by a plastic film.

Plants were grown and irrigated daily for 25 d. From 5 to 25 DAI, five plants per treatment were collected every 5 d for analysis of nematode penetration and development. Briefly, the root system was carefully separated from shoots, washed, placed on absorbent paper to remove excess water, and weighed to obtain the root fresh weight (g). Then, root fragments were stained with acid fuchsin (Byrd Junior et al., 1983). Temporary slides were prepared using all root fragments from each plant and observed under a light microscope at 100X magnification. *Meloidogyne javanica* individuals were counted and classified according to developmental stages into J2, third-stage juveniles (J3), fourth-stage juveniles (J4), and adult females. The total number of nematodes and number of nematodes per gram of root (population density) were also determined.

Experiment II. Influence of *A. nodosum* extract and its nutrient components on the control of *M. javanica* in soybean

The second experiment was conducted from March to May 2021 (Trial 1), with mean minimum, maximum, and average temperatures of 23.8, 25.2, and 24.5 °C, respectively, and repeated from April to June 2021 (Trial 2), with temperatures of 22.4, 21.2, and 21.8 °C, respectively. The design was completely randomized with six replicates of 11 treatments: *A. nodosum*, N, K, P, Ca, Mg, Cu, Zn, Mn, Fe, combined nutrients, and untreated control. Nutrients and their rates were determined from the chemical composition of the algal product (Table 1). In each experimental unit, a 4 cm deep hole was opened in the soil and treatments were applied by sprinkling into the planting hole. Subsequently, 1 seed of soybean was sown and inoculated with 2000 eggs+J2 of the nematode.

At 60 DAI, plants were harvested and separated into shoots and roots. The root system was washed and weighed, and the gall index was determined (Taylor and Sasser, 1978). Then, roots were subjected to the above-mentioned process for nematode extraction. Total nematode number was determined and divided by root fresh weight to obtain nematode population density. Shoots were measured with a millimeter ruler (cm) to obtain plant height. Shoot fresh and dry weights were determined using a semi-analytical balance. For dry weight determination, shoots were placed in paper bags and dried in a forced-air oven at 65 °C until constant weight was achieved.

Statistical analysis

Data were assessed for normality of distribution and homogeneity of variance by Shapiro-Wilk and Levene's tests, respectively. When necessary, the data were transformed to $\sqrt{x}$ before analysis. Data from Experiment 1 were subjected to two-way ANOVA to assess the effects of sampling time, method of extract application, and their interactions on nematode development and final populations ($p < 0.05$). Differences between means were compared by Tukey's test ($p < 0.05$). Data from Experiment 2 were subjected to one-way ANOVA to investigate treatment effects on nematode and vegetative variables. Means were compared by the Scott-Knott test ($p < 0.05$). All statistical analyses were performed using Sisvar software (Ferreira, 2011).

RESULTS

Experiment I. Penetration and development of *M. javanica* in soybean roots treated with *A. nodosum* extract

There was no interaction between sampling time and method of application on J2 penetration into soybean roots. However, the main effects of method of application were significant. Planting hole and foliar applications reduced J2 penetration (Table 2).

Table 2. Numbers of second-stage (J2), third-stage (J3), and fourth-stage (J4) juveniles, adult females, total nematodes, and nematodes per gram of root in soybean crops treated with *Ascophyllum nodosum* by different application methods. Means within columns followed by the same lowercase letter and means within rows followed by the same uppercase letter are not significantly different by Tukey's test ($p < 0.05$). DAI: Days after inoculation; CV: coefficient of variation.

Treatment	5 DAI	10 DAI	15 DAI	20 DAI	25 DAI	Mean
			J2			
Planting hole	5.0	7.3	6.3	15.0	15.3	9.8b
Foliar spraying	0.6	7.0	32.0	10.6	4.6	11.0b
Control	25.6	43.0	90.6	108.6	27.0	59.0a
Mean	10.4B	19.1AB	43.0A	44.7A	15.6AB	CV = 49.3%
			J3			
Planting hole	0.0aA	0.0aA	0.0bA	2.3bA	0.6bA	0.6
Foliar spraying	0.0aB	0.0aB	27.3aA	15.6aA	0.6bB	8.7
Control	0.0aB	9.6aAB	9.0aAB	14.6aA	13.6aA	9.4
Mean	0.0	3.2	12.1	10.8	5.0	CV = 87.0%
			J4			
Planting hole	0.0aA	0.0aA	0.0aA	0.0bA	3.3bA	0.6
Foliar spraying	0.0aB	0.0aB	3.0aAB	18.3aA	1.6bB	4.6
Control	0.0aB	1.6aB	5.0aB	12.6abB	28.3aA	9.5
Mean	0.0	0.5	2.5	10.3	11.1	CV = 107.2%
	Adult females					
Planting hole	0.0aA	0.0aA	0.0aA	0.0aA	0.6bA	0.1
Foliar spraying	0.0aA	0.0aA	0.0aA	1.2aA	1.2bA	0.6
Control	0.0aB	0.0aB	2.0aB	3.3aB	12.0aA	2.8
Mean	0.0	0.0	0.6	1.1	4.2	CV = 121.5%
			Total nematodes			
In-hole application	5.0	17.0	6.3	17.3	20.0	13.1b
Foliar application	0.6	7.0	62.3	48.0	7.0	25.0b
Control	25.6	54.3	106.6	136.0	81.0	80.7a
Mean	10.4B	26.1AB	58.0A	67.0A	36.0AB	CV = 45.3%
			Nematodes g^{-1} root			
Planting hole	3.3	10.8	4.3	33.8	82.5	26.9b
Foliar spraying	2.4	4.0	57.5	15.5	13.2	18.5b
Control	44.9	59.9	60.4	112.8	26.4	60.9a
Mean	16.9A	24.9A	40.7A	54.0A	40.7A	CV = 66.4%

Interaction effects were significant for J3 and J4 counts. The lowest J3 number was obtained with hole application. No J3 individuals were detected up to 20 DAI in plants treated with *A. nodosum* extract in the planting hole. In plants treated by foliar spraying, the beginning of this developmental stage as well as the highest number of J3 individuals were observed at 15 DAI, whereas, in the control, J3 formation occurred from 10 DAI onward (Table 2). In plants of the planting hole treatment, J4 formation started at 25 DAI. By contrast, in plants treated by foliar application, J4 formation was first observed at 15 DAI, peaked at 20 DAI, and decreased at 25 DAI. In control plants, J4 individuals were detected at 10 DAI, and the highest numbers were recorded at 25 DAI. In-hole treatment provided the lowest J4 number (Table 2).

Significant interaction effects were observed on female number, with lower numbers in plants treated with algal extract, regardless of the method of application. Females were detected from 25 DAI onward in plants subjected to in-hole treatment and from 20 DAI onward in plants treated by foliar spraying. In the control, females were detected in the roots from 15 DAI onward, with a peak at 25 DAI, when numbers were significantly higher than in the other treatments (Table 2).

Finally, there were no interaction effects on total nematode number or population density. However, the main effects of application method were significant. In-hole and foliar spraying treatments significantly reduced total nematode number and population density compared with the control (Table 2).

Experiment II. Influence of *A. nodosum* extract and its nutrient components on *M. javanica* control in soybean

Nitrogen (2.0) and P (1.8) were the most efficient in reducing gall index. By contrast, K (4.6), Cu (4.0), Zn (4.0), Mn (4.0), Fe (3.6), and combined nutrients (4.3) increased this variable compared with the control (2.6) in Trial 1 (Table 3). All treatments, except Cu, reduced total nematode number in Trial 1 compared with the control, with P, Mg, and Fe being the most efficient; these nutrients afforded reductions of 92%, 95%, and 92%, respectively (Table 3). Regarding nematode population density, only *A. nodosum* extract and Cu did not differ from the control. The highest reductions in this variable (83%-95%) were afforded by P, Ca, Mg, Fe, and combined nutrients (Table 3).

Table 3. Gall index, total nematode number, and number of nematodes per gram of root in soybean crops treated with nutrients or a commercial fertilizer based on *Ascophyllum nodosum* (Experiment 2, Trials 1 and 2). Means within columns followed by the same letter are not significantly different by the Scott-Knott test ($p < 0.05$). Original data were transformed to $\sqrt{x}$ before analysis. CV: Coefficient of variation.

	Gall index		Total nematode number		Nematodes g^{-1} root	
Treatment	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	2.6b	2.2^{ns}	15068a	14376a	3698a	5393a
A. nodosum	2.8b	2.2	7098b	5670b	2632a	936b
N	2.0c	2.8	4219c	9813a	1178b	4232a
K	4.6a	3.0	7843b	12111a	1207b	3066a
P	1.8c	1.8	1180d	6910a	488c	1713b
Ca	3.0b	2.2	2791c	3513b	628c	702b
Mg	3.0b	2.3	721d	4736b	174c	1283b
Cu	4.0a	3.5	14978a	8753a	2819a	1660b
Zn	4.0a	2.8	3785c	5495b	921b	1372b
Mn	4.0a	2.2	4386c	847b	941b	392b
Fe	3.6a	1.8	1108d	6923a	315c	2057b
Combined nutrients	4.3a	2.0	3184c	2134b	540c	857b
CV, %	10.99	17.65	36.89	39.67	37.24	42.23

In Trial 2, there were no differences in gall index between treatments, and total nematode number was negatively affected by treatments (Table 3) *Ascophyllum nodosum* extract, Ca, Mg, Zn, Mn, and combined nutrients reduced *M. javanica* number by 60% to 94% (Table 2). All treatments, except N and K, differed in population density from the control, with a control efficiency of 61% to 92% (Table 3).

There were no differences in root fresh weight, plant height, or shoot fresh weight in Trial 1. Copper, Mn, and combined nutrients afforded the highest shoot dry weights (Table 4). In Trial 2, root fresh weight was significantly higher in plants treated with *A. nodosum* extract, Ca, and Cu. Furthermore, plant height was significantly increased by all treatments, except Mg and combined nutrients (Table 4). *Ascophyllum nodosum* extract, P, Ca, Mg, Cu, Zn, and Fe provided the highest shoot fresh weights. Shoot dry weight was not influenced by treatment (Table 4).

Table 4. Root fresh weight (RFW), plant height, shoot fresh weight (SFW), and shoot dry weight (SDW) of soybean treated with nutrients or a commercial fertilizer based on *Ascophyllum nodosum* (Experiment 2, Trials 1 and 2). Means within columns followed by the same letter are not significantly different by the Scott-Knott test ($p < 0.05$). Original data were transformed to $\sqrt{x}$ before analysis. CV: Coefficient of variation; ns: nonsignificant.

	RFW		Plant height		SFW		SDW	
Treatment	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
	g		cm		g		g	
Control	4.56[ns]	2.90b	28.51[ns]	20.50b	8.88[ns]	2.86b	3.01b	0.79[ns]
A. nodosum	4.32	5.81a	29.31	23.58a	7.38	5.11a	2.31b	1.43
N	3.08	3.28b	25.08	23.80a	7.12	3.13b	2.47b	0.96
K	6.20	3.94b	26.93	23.91a	9.19	3.03b	2.91b	0.81
P	3.25	4.19b	25.80	24.78a	6.12	5.81a	1.93b	1.18
Ca	4.22	5.24a	25.80	26.33a	7.56	6.82a	2.31b	1.72
Mg	4.20	4.08b	27.41	25.45a	8.19	4.26a	2.69b	1.16
Cu	5.23	5.93a	31.50	23.50a	11.28	5.14a	4.09a	1.28
Zn	4.10	3.87b	26.08	25.33a	7.96	4.11a	2.55b	0.97
Mn	5.36	2.88b	34.88	18.80b	12.30	2.69b	4.53a	0.60
Fe	3.84	3.52b	29.13	22.83a	9.31	4.20a	2.98b	1.11
Combined nutrients	5.91	2.56b	27.66	21.80b	10.36	2.62b	3.36a	0.64
CV, %	22.12	20.12	10.32	7.49	21.74	22.67	18.42	18.45

DISCUSSION

Ascophyllum nodosum aqueous extract was efficient in reducing the total number and population density of *M. javanica* in soybean. Furthermore, we observed a delay in nematode development with extract application. Previous studies reported on the effectiveness of this alga in controlling nematodes in several plant species (Radwan et al., 2012; El-Deen et al., 2013; Ngala et al., 2016; Rinaldi et al., 2021).

The results of the current study are in agreement with those of Rinaldi et al. (2021), who treated soybean plants with different concentrations of *A. nodosum* extract via different application modes. The authors found that *M. javanica* reproduction was suppressed in treated plants. Furthermore, hatching was inhibited by 92% using minimal doses of 29.5 and 30.3 g L^{-1}. Studies assessing the development of *M. incognita* in tomato crops found that the algal extract reduced J2 number in soil and gall number in roots compared with the control (Radwan et al., 2012). Similarly, algal extract treatment of basil plants infected with *M. incognita* led to a reduction in gall index and egg mass number (El-Deen et al., 2013).

Several marine algae have been studied for their effects on phytonematodes; *A. nodosum*, in particular, has shown superior nematicidal effects on *Meloidogyne* spp. compared with other algae (Williams et al., 2021). The efficiency of *A. nodosum* in the control of *Meloidogyne* spp. might be related to the presence of betaines, ammonium compounds that act physiologically as cytoplasmic osmotic regulators and may play a role as modulators under situations of biotic and abiotic stress (Blunden, 2003). These formaldehyde precursors are associated with inducing disease resistance in plants (Jenkins et al., 1998), protecting cells, proteins, and enzymes and thereby minimizing stress-induced damage resulting from parasitism (Wu et al., 1997). The algal extract also contains alginates, which may act as elicitors of plant defense through the salicylic acid signaling pathway (Ali et al., 2019).

Compounds related to resistance induction in plants are commonly found in marine algal extracts, including proteins, glycoproteins, peptides, polysaccharides, oligosaccharides, and lipids (Klarzynski et al., 2000). Algal extracts have several bioactive organic and inorganic components found in plants, such as mannitol, laminarin, alginates, vitamins, antioxidants, phytohormones (auxins, cytokinins, gibberellins, and betaines), and minerals (K, P, Ca, B, Mg, and Zn, among others) (Klarzynski et al., 2000).

Rinaldi et al. (2021) reported the induction of resistance to nematodes in soybean by treatment with *A. nodosum* extract via foliar spraying and in-hole application. The extract was found to suppress *M. javanica* hatching and reproduction on soybean, as well as increase the activity of enzymes related to soybean defense, such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and β-1,3-glucanase.

It is noteworthy that the defense and tolerance mechanisms of plants against pathogen attack are influenced by various factors, including mineral nutrition. The higher resistance and tolerance of well-nourished plants against diseases is due to the fact that vigorous, healthy plants have a greater capacity to compensate, even if partially, for losses caused by pathogens, including nematodes; in some cases, such responses may stimulate plant development (Santana-Gomes et al., 2013).

In the present study, it was demonstrated that nutrients found in *A. nodosum* extract, whether isolated or combined, negatively affected at least one nematode parameter in soybean plants infected with *M. javanica*. Previous research demonstrated the effectiveness of fertilizers in the management of root-knot nematodes and promotion of plant growth and productivity (Akhtar et al., 2013). Application of K to different crops, for example, led to reductions in nematode infection, an increase in the activity of enzymes related to resistance, such as catalase, peroxidase, and phenylalanine ammonia-lyase, and improvement in phenol and flavonoid levels (Zhao et al., 2016). In addition to playing an important role in the activation of more than 60 enzymes, adequate K nutrition may help reduce the incidence of diseases, as a result of increased resistance to pathogen penetration and development (Santana-Gomes et al., 2013).

In this study, fertilization with the macronutrients N and P reduced gall index in Trial 1, corroborating a previous study reporting a decrease in gall index in cucumber treated with N, P, and K, as well as an increase in vegetative parameters and crop yield (Mansourabad et al., 2016). Nitrogen in the form of ammonium, present in fertilizers and organic matter, is more harmful to nematodes than N in the form of nitrate, as the former releases free ammonia into the soil. Urea has been widely investigated for this purpose, as it is rapidly converted to ammonia, which exerts nematicidal effects, by the action of urease in the soil.

Phosphorus is known to contribute to root growth and absorption capacity (Hussey and Roncadori, 1982); however, the compound also stimulates protein synthesis, cellular activity, the release of fewer root exudates, and synthesis of polyphenols, peroxidase, and ammonia, influencing plants and nematodes (Marschner, 2012).

Calcium and Mg treatments, despite affording variable results, provided a reduction in *M. javanica* populations. Calcium is essential for the integrity of the plasma membrane of plants; it plays a role in several processes, such as formation of the middle lamella (Marschner, 2012). Application of a Ca-based product reduced the number of galls, egg masses, and juveniles of *M. incognita* in *Cucurbita pepo* and increased crop yield, regardless of the concentration used (Mohamed and Youssef, 2009).

Together with macronutrients, micronutrients play an important role in plant responses against pathogens. Micronutrients not only provide nutritional benefits but also act as enzyme cofactors (Marschner, 2012). Foliar application of Zn was found to reduce the number of eggs and galls of *M. incognita* in tomato (Couto et al., 2016). The referred authors hypothesized that micronutrients such as Zn can improve the efficiency of resistance inducers and that balanced fertilization can be used as part of an integrated management strategy for the control of *M. javanica* in soybean. Isolated Zn was effective in reducing *Pratylenchus brachyurus* in soybean, but the best result was obtained by combination with phosphorylated mannanoligosaccharide derived from the cell wall of *Saccharomyces cerevisiae* (MOS) (Conduta et al., 2020).

Manganese, which reduced *M. javanica* populations in both trials, was previously reported as an effective compound for the control of *P. brachyurus*, whether isolated or combined with a MOS-based product (Conduta et al., 2020). Possibly, the suppressive action of Mn on nematodes is related to the compound's capacity of activating enzymes responsible for the production of secondary metabolites, such as lignin and flavonoids (Barker and Pilbeam, 2007). According to the authors, lignification may increase cell wall rigidity and thus negatively influence feeding site development (Melillo et al., 2014). Copper, in a similar manner, provides ions that act as cofactors of several enzymes found in plant cells, influencing the formation and composition of the cell wall (Marschner, 2012). As a result, toxic or physical barriers are formed, impairing pathogen penetration, as seen in pathosystems involving fungi (Bulhões et al., 2012).

Ascophyllum nodosum extract had a positive effect on plant height and shoot fresh weight. Similar results were obtained with nutrient application, corroborating previous research (Rinaldi et al., 2021). The findings suggest that bioactive substances present in the algal extract promote shoot development, root and leaf growth, and nutrient absorption, contributing to overall plant growth (Fan et al., 2011).

Overall, the results showed that *A. nodosum* modulates the amount of endogenous plant hormones, contributing to nutrient balance, regulation of nutrient absorption and assimilation, stimulation of photosynthesis, and mitigation of stress caused by parasitism (De Saeger et al., 2020). It is not possible to attribute the positive results of *A. nodosum* extract to certain constituents only, given the variety of molecules that perform various functions in complex, dynamic processes. Thus, it is difficult to discriminate between primary and secondary effects.

Although there are reports of direct control of nematodes by *A. nodosum*, mostly in in vitro experiments, it has been previously suggested that the effects of the alga on pathogen control are indirect. Absorption of the extract by plants leads to compound synthesis and activation of pathways responsible for reducing nematode populations (Wu et al., 1997). Thus, it is believed that, in addition to the biostimulating effect of *A. nodosum* in plants, nematode suppression is achieved by the action of bioactive molecules found in the extract, which act together and trigger various metabolic processes, including resistance induction. Furthermore, the algal extract supplies nutrients to plants, which may contribute to the activation of defense-related enzymes. More research is needed on the influence of mineral nutrition on nematode control and on the mechanisms of action of nutrients against pathogens.

The results of the current study demonstrated that *A. nodosum* extract has potential for the management of *M. javanica* in soybean, as it reduces and delays nematode penetration and development and stimulates vegetative growth. Additional studies are needed to validate the use of *A. nodosum* extract for the control of phytonematodes in the field and determine its economic viability.

CONCLUSIONS

In-hole and foliar application of *Ascophyllum nodosum* extract reduced *Meloidogyne javanica* penetration and development in soybean. All nutrients had positive effects on the control of *M. javanica*, reducing at least one nematode variable; however, Ca, Mg, Zn, Mn, and combined nutrients reduced nematode populations in both trials. All nutrients were efficient in increasing at least one vegetative parameter in both trials.

Author contributions

Conceptualization: L.K.R., C.R.D-A. Methodology: L.K.R., C.R.D-A. Software: L.K.R., C.R.D-A. Validation: L.K.R. Formal analysis: L.K.R. Investigation: L.K.R., A.C., A.M., M.T.R.S. Resources: C.R.D-A. Data curation: L.K.R. Writing-original draft: L.K.R. Writing-review & editing: L.K.R., A.C., A.M., M.T.R.S. Visualization: L.K.R., A.C., A.M., M.T.R.S. Supervision: L.K.R., C.R.D-A. Project administration: L.K.R., C.R.D-A. Funding acquisition: L.K.R., C.R.D-A. All co-authors reviewed the final version and approved the manuscript before submission.

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