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Control of *Meloidogyne javanica* and induction of resistance-associated enzymes in soybean by extracts of *Ascophyllum nodosum*

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Abstract

This study investigated the ability of an aqueous extract of *Ascophyllum nodosum* to directly control the root-knot nematode *Meloidogyne javanica* and induce nematode resistance in soybean. In a first experiment, soybean plants were inoculated with 2000 eggs and juveniles of *M. javanica* and treated with extract doses of 0 (control), 25, 50, 75, and 100 g L^{-1} by two methods of application, soil drenching and foliar spraying. A second experiment evaluated the effect on the hatching percentage of 500 *M. javanica* eggs. A third experiment assessed defense enzyme activity in inoculated and uninoculated plants treated with *A. nodosum* extract (75 g L^{-1}) by soil drenching or foliar spraying. This experiment was performed once and analyzed in duplicate. Inoculated and uninoculated untreated plants were included as controls. High extract doses (75 and 100 g L^{-1}) promoted a 65% reduction in nematode population density in soybean. At doses of 32 g L^{-1} or higher, extract application by soil drenching increased shoot dry weight and plant height. The minimum dose of algal extract to obtain hatching percentages close to 0% was 21.8–22.2 g L^{-1}. Peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and glucanase activities were highest in uninoculated drench-treated plants at 12 days after treatment. *A. nodosum* extract was effective in reducing *M. javanica* population density in soybean and in vitro egg hatching. The use of the extract also offered potential to the increase in the activity of enzymes related to plant defense in relation to inoculated and untreated control and the uninoculated and untreated control.

Keywords *Glycine max* · Resistance enzyme · Root-knot nematode · Seaweed

Introduction

Ascophyllum nodosum (L.) Le Jolis is a brown perennial seaweed that is common along the coast of temperate countries, such as Canada, France, Ireland, Iceland, Norway, and the UK (Kandasamy et al. 2011; Bozorgi 2012). This alga has been extensively researched because of its ability to promote plant growth (Fan et al. 2014). *Ascophyllum nodosum* extract is used commercially in agriculture as foliar spray and soil amendment (Khan et al. 2009; Igna and Marchioro 2010). About 30,000 tonnes of *A. nodosum* are used in the manufacture of agricultural products each year (Craigie 2011). *Ascophyllum nodosum* extract contains biologically active compounds, including macro- and micronutrients, polyphenols, betaines, polysaccharides, fatty acids, steroids, polyamines, and analogs of the phytohormones auxin, cytokinin, gibberellin, and abscisic acid (Craigie 2011; Sangha et al. 2014). The extract has been shown to promote plant resistance to biotic and abiotic stresses and increase soil fertility (Khan et al. 2009; Alan et al. 2013).

Several studies have reported the potential of seaweed extracts to protect plants and plant organs against phytopathogens, for instance, by reducing the severity of fungal diseases caused by *Botrytis cinerea* and *Alternaria radicina* in carrot (Jayaraj et al. 2008) and *Alternaria cucumerina*, *Didymella applanata*, *Fusarium oxysporum*, and *B. cinerea* in cucumber (Jayaraman et al. 2011). More specifically, brown algal exracts have been used to control nematode infection in plants (Paracer et al. 1987; Zaki et al. 2005). Betaines, found in alkaline extracts of brown algae, were effective in reducing the fecundity of *Meloidogyne javanica* and *M. incognita* in

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tomato (Wu et al. 1997). Brown algal extracts killed *M. javanica* juveniles in vitro (Rizvi and Shameel 2006) and, when applied to the soil, decreased the number of eggs of *M. chitwoodi* and *M. hapla* in tomato (Ngala et al. 2016). *Ascophyllum nodosum* is a prominent example of brown algae with the ability to promote plant growth (Khan et al. 2009) and induce plant resistance to pathogens such as nematodes (Featonby-Smith and Van Staden 1983).

Sulfated and nonsulfated complex polysaccharides are commonly found in algal cell walls. These compounds have diverse biological activities, including, for instance, elicitation of plant defense responses (Mercier et al. 2001; Rahman et al. 2017). Seaweed extracts enhanced the production of the phytoalexin capsidiol and increased peroxidase (POX) activity in pepper and grapevine (Lizzi et al. 1998). The mechanism of action of algal extract in inducing resistance against *Pseudomonas syringae* pv. tomato DC3000 and *Sclerotinia sclerotiorum* in *Arabidopsis thaliana* was associated with activation of the jasmonic acid pathway (Subramanian et al. 2011). This pathway depends on jasmonic acid and ethylene for molecular signaling and is generally activated by nonpathogenic microorganisms; therefore, it is not directly involved in the accumulation of pathogenesis-related proteins (Choudhary et al. 2007; Pieterse et al. 2014). Algal polysaccharides were also shown to promote local production of chitinase and proteinase inhibitors and activate salicylic acid pathways, which are involved in systemic acquired resistance responses (Mercier et al. 2001).

Considering the benefits of algal extracts to plant growth, this study hypothesized that brown algal extract can be part of an integrated and sustainable management of nematodes in soybean (*Glycine max* (L.) Merrill), contributing to minimizing the large-scale use of synthetic chemicals. The aim was to investigate the potential of *A. nodosum* extract to directly control *M. javanica* and induce nematode resistance in soybean.

Materials and methods

General experimental procedures

In vivo experiments were conducted in a greenhouse (23° 24′ 17″ S 51° 56′ 26″ W, 313 m elevation) and in vitro experiments at the laboratory (23° 24′ 18″ S 51° 56′ 29″ W, 313 m elevation). For greenhouse trials, the experimental units were soybean cv. M6210 IPRO plants transplanted to polystyrene cups (13.8 cm high and 9 cm in diameter). Cups contained 0.5 L of autoclaved (120 °C, 2 h) soil and sand at a ratio of 1:2. Plants were irrigated daily as needed. For in vitro experiments, each tube was treated as an experimental unit.

A commercial water-soluble powdered product based on *A. nodosum*, soluble in water (Ativa Power®) was obtained from Alternativa Agrícola (Mogi Guaçu, SP, Brazil). According to chemical analysis, the product contains 10.42 g kg^{-1} nitrogen, 0.66 g kg^{-1} phosphorus, 142.44 g kg^{-1} potassium, 3.95 g kg^{-1} calcium, 2.11 g kg^{-1} magnesium, 20.67 mg kg^{-1} copper, 3591.76 mg kg^{-1} iron, 28.42 mg kg^{-1} manganese, and 37.47 mg kg^{-1} zinc. Doses of 0, 2.5, 5.0, 7.5, and 10 kg ha^{-1} (equivalent to concentrations of 0, 25, 50, 75, and 100 g L^{-1}, respectively) were prepared by mixing the extract with distilled water to provide an application volume of 100 L ha^{-1}. Extract dilutions were homogenized on a shaker for 24 h and stored for later use.

The nematode inoculum was obtained from a pure population of *M. javanica* maintained on tomato cv. Santa Clara under greenhouse conditions. For extraction of eggs and second-stage juveniles (J2), plant roots were collected and subjected to the method of Hussey and Barker (1973) as modified by Bonetti and Ferraz (1981). Nematodes were counted using a Peters' chamber under an optical microscope, and the inoculum size was adjusted to 2000 eggs + J2 mL^{-1} for in vivo experiments and 500 eggs mL^{-1} for in vitro assays.

Effect of *A. nodosum* extract on *M. javanica* population density and soybean development

The experiment was conducted from March to April 2019 (Trial 1) and repeated from September to October 2019 (Trial 2). Treatments followed a completely randomized design arranged in a 5 × 2 factorial, with five extract doses (0, 25, 50, 75, and 100 g L^{-1}), two methods of application (soil drenching and foliar spraying), and six replications. Distilled water was used as control.

Before drench application, a 4 cm deep hole was made in the soil of each pot. Extract doses were applied directly into the furrow by spraying. Then, soybean seedlings at the V2 stage, grown in polystyrene trays, were transplanted to the pots and inoculated with 2000 *M. javanica* eggs + J2 in two equidistant holes in the soil surrounding seedling roots. For plants in the foliar treatment group, extract doses were applied immediately after transplanting and inoculation. Leaves were sprayed to the point of runoff while the soil around the seedling was protected with plastic film. Drench and foliar treatments were reapplied 20 days later.

At 45 days after transplanting, plants were harvested and separated into shoots and roots. Shoot height was measured using a millimeter ruler, and shoot fresh and dry weights were determined on 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.

Roots were thoroughly washed and blotted dry with paper towels to remove excess water. Then, the root fresh weight and gall index were determined (Taylor and Sasser 1978). Roots were subjected to nematode extraction (Hussey and Barker 1973; Bonetti and Ferraz 1981), and total nematode

number and number of nematodes per gram of root (population density) were determined.

Effect of *A. nodosum* extract on *M. javanica* egg hatch

The experiment was conducted in duplicate following a completely randomized design, with five treatments and six replications. Briefly, 1 mL of a suspension of 500 *M. javanica* eggs and 4 mL of *A. nodosum* extract at different concentrations (0, 25, 50, 75, and 100 g L^{-1}) were added to polypropylene Falcon tubes, totaling 5 mL each. Distilled water was used as negative control. Tubes were incubated in a biochemical oxygen demand incubator (340 L, − 10 to 60 °C, NI1705, Nova Instruments) at 27 °C for 10 days and shaken manually for 2 min three times a day. The hatching percentage was determined by counting the number of hatched juveniles and unhatched eggs using a Peters' counting chamber and an optical microscope.

Induction of enzymes associated with plant resistance against *M. javanica* in soybean

This experiment, installed as described above for the nematode reproduction and soybean development test, was carried out from September to November 2019. The experimental design was completely randomized with a 6 × 3 factorial arrangement. The first factor comprised the following treatments: (i) inoculated and (ii) uninoculated plants treated with *A. nodosum* extract by soil drenching, (iii) inoculated and (iv) uninoculated plants treated by foliar spraying, (v) inoculated untreated plants, and (vi) uninoculated untreated plants. The second factor was the evaluation period: 4, 8, and 12 days after treatment (DAT).

Soybean seedlings were grown in polystyrene trays containing potting substrate (Plantmax) until reaching the V2 stage. Then, the plants were transplanted, treated, and inoculated as described for the previous experiment, except that the extract dose was fixed at 75 g L^{-1}. At each evaluation period, about 0.5 g of leaf tissue (from the third partially expanded leaf) was collected, placed in aluminum foil envelopes, and stored at − 80 °C until analysis.

For enzyme extraction, specimens were ground in liquid nitrogen using mortar and pestle and received the addition of 1% (v/v) polyvinylpyrrolidone and 4 mL of 50 mM potassium phosphate buffer containing 0.1 mM EDTA (pH 7.0). Samples were centrifuged at 14,000 rpm and 4 °C for 30 min. The supernatant was transferred to 1.5 mL microtubes, stored in a freezer at − 80 °C, and used as enzyme extract. The experiment was performed once and analyzed in duplicate.

Peroxidase (POX, EC 1.11.1.7) activity was estimated by measuring the conversion of guaiacol to tetraguaiacol in the presence of hydrogen peroxide (Lusso and Pascholati 1999). Enzyme extract (100 μL) was mixed with 2.9 mL of substrate (7.25 μL of guaiacol and 8 874 μL of H_2O_2 diluted in 50 mM potassium phosphate buffer, pH 7.0) and kept in a water bath at 30 °C. Absorbance was read spectrophotometrically at 470 nm, and results are expressed as change in absorbance min^{-1} mg^{-1} protein.

β-1,3-Glucanase (GLU, EC 3.2.1.6) activity was determined by quantifying the amount of glucose released by hydrolysis of laminarin (Vogelsang and Barz 1993). First, 150 μL of enzyme extract and 150 μL of 2 mg mL^{-1} laminarin were added to a test tube and incubated at 40 °C for 1 h. A control was prepared by adding laminarin only after incubation. Subsequently, 50 μL aliquots were transferred to a new test tube, mixed with 1.5 mL of 0.5% 4-hydroxybenzoic acid hydrazide in 0.5 M NaOH, incubated at 100 °C for 5 min, and cooled in an ice bath for 2 to 3 min (Lever 1972). Absorbance was measured spectrophotometrically at 410 nm. Concentrations were determined against a standard curve of glucose, and results are expressed in mg glucose h^{-1} mg^{-1} protein.

For determination of polyphenol oxidase (PPO, EC 1.10.3.2) activity, 100 μL of the enzyme extract was mixed with 900 μL of 20 mM catechol in 0.1 M potassium phosphate buffer (pH 6.8) in a beaker. Samples were covered with aluminum foil and incubated in a water bath at 30 °C. Absorbance readings were performed at 420 nm. The oxidation of catechol to quinone was expressed as change in absorbance min^{-1} mg^{-1} protein (Duangmal and Apenten 1999).

Determination of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity was performed according to the method described by Umesha (2006). Enzyme extract (100 μL) was mixed with 400 μL of 0.025 M Tris-HCl buffer (pH 8.8) and 500 μL of 0.05 M L-phenylalanine solution (825.9 mg of L-phenylalanine diluted in 100 mL of 0.025 M Tris-HCl buffer, pH 8.8). The mixture was incubated in a water bath at 40 °C for 1 h. Then, 60 μL of 5 M HCl was added to stop the reaction, and samples were analyzed spectrophotometrically at 290 nm. PAL activity was calculated as the difference in absorbance between sample and blank (prepared without L-phenylalanine). Concentrations were read against a standard curve of *trans*-cinnamic acid, and results are expressed as mg *trans*-cinnamic acid h^{-1} mg^{-1} protein.

Statistical analysis

Data were subjected to analysis of variance and, when significant, means were compared by the Scott–Knott test at $p < 0.05$. Values were square-root transformed to meet normality assumptions, as assessed by the Shapiro–Wilk test. Analyses were performed using Sisvar (Ferreira 2011).

A segmented quadratic model with plateau was fitted to in vitro egg hatch data using the easyreg package of R version 3.6.3 (R Core Team 2020). For the resistance induction experiment, means were compared by the Scott–Knott test at $p < 0.05$ using Sisvar (Ferreira 2011).

Results

Effect of *A. nodosum* extract on *M. javanica* and soybean development

In Trial 1, total nematode number decreased as a function of algal extract dose. Nematode control was most effective when using 50, 75, and 100 g L^{-1} algal extract. No differences were observed between application methods, regardless of the dose (Table 1). In Trial 2, all doses were efficient, reducing total nematode number by about 38% compared with the control. Application method did not influence total nematode number (Table 1).

In Trial 1, nematode population density decreased by about 68% at all tested doses compared with the control. No differences were found between application methods (Table 1). In Trial 2, neither dose nor application method exerted significant effects on nematode population density (Table 1).

Significant interaction effects of dose × application method on gall index were observed in Trial 1. At doses of 25, 75, and 100 g L^{-1}, the extract reduced gall index when applied as soil drench. When used as foliar spray, the extract caused a significant reduction in gall index at a dose of 100 g L^{-1}. At 75 g L^{-1}, the extract was more effective as foliar spray than as soil drench (Table 1). In Trial 2, gall index was significantly influenced by dose only. The highest reduction in gall index was achieved using a dose of 50 g L^{-1}. No differences were found between application methods (Table 1).

In Trial 1, shoot fresh weight was higher in treated plants than in the control, not differing between doses. Application method had no significant effect on the parameter (Table 2). In Trial 2, no differences were observed between doses or application methods (Table 2). Shoot dry weight was not influenced by either parameter in both trials (Table 2).

Extract dose had a significant influence on plant height in Trial 1. The largest reduction was observed when using 75 g L^{-1} extract. Means were not influenced by application method (Table 2). In Trial 2, no differences were observed between doses or application methods (Table 2).

Table 1 Total nematode number, nematode population density, and gall index in soybean inoculated with 2000 eggs + second-stage juveniles of *Meloidogyne javanica* and treated with an aqueous extract of *Ascophyllum nodosum* applied as soil drench or foliar spray (FS) in two trials

Total nematode number						
Dose (g L^{-1})	Trial 1			Trial 2		
	Drench	FS	Mean	Drench	FS	Mean
0	118,583	101,526	110,055 a	30,023	35,451	32,737 a
25	86,258	92,209	89,233 a	21,431	18,750	20,091 b
50	53,395	78,681	66,038 b	16,337	23,785	20,061 b
75	38,888	50,202	44,545 b	16,783	20,663	18,723 b
100	74,408	65,110	69,759 b	21,724	20,710	21,217 b
Mean	74,306^{ns}	77,546		21,260^{ns}	23,872	
CV (%)	26.71			25.74		
Nematode population density (nematodes g^{-1} root)						
0	68,145	28,717	48,431 a	5081	5781	5431^{ns}
25	26,430	17,603	22,017 b	3776	4241	4008
50	9916	15,706	12,811 b	3687	5183	4435
75	10,113	14,835	12,474 b	2629	3899	3264
100	14,173	14,718	14,445 b	4018	4477	4248
Mean	25,755^{ns}	18,316		3838^{ns}	4716	
CV (%)	40.61			27.73		
Gall index						
0	5.0 aA	4.8 aA	4.9 a	4.5	4.0	4.2 a
25	4.0 bA	4.3 aA	4.2 b	4.3	4.3	4.3 a
50	4.6 aA	4.5 aA	4.6 a	2.6	3.5	3.1 b
75	3.5 bB	4.6 aA	4.1 b	4.1	4.0	4.1 a
100	3.8 bA	3.6 bA	3.7 b	3.8	3.3	4.1 a
Mean	2.04 A	2.08 A		3.9^{ns}	4.0	
CV (%)	6.57			12.27		

Means followed by the same lowercase letter within a row and uppercase letter within a column do not differ by the Scott–Knott test at the 5% significance level. *CV*, coefficient of variation; *ns*, not significant

Table 2 Shoot fresh weight (SFW), shoot dry weight (SDW), and height of soybean plants inoculated with 2000 eggs + second-stage juveniles of *Meloidogyne javanica* and treated with an aqueous extract of *Ascophyllum nodosum* applied as soil drench or foliar spray (FS) in two trials

SFW (g)						
Dose (g L^{-1})	Trial 1			Trial 2		
	Drench	FS	Mean	Drench	FS	Mean
0	11.26	10.14	10.70 b	4.30	4.87	4.58^{ns}
25	12.21	12.53	12.37 a	4.24	4.17	4.18
50	12.44	12.47	12.45 a	5.17	4.74	4.95
75	12.49	11.77	12.13 a	4.87	4.67	4.75
100	12.66	11.74	12.20 a	3.53	4.34	3.93
Mean	12.21^{ns}	11.73		4.42^{ns}	4.54	
CV (%)	22.09			25.88		
SDW (g)						
0	5.22	4.74	4.98^{ns}	1.61	1.94	1.77^{ns}
25	3.68	5.63	4.65	1.96	1.54	1.75
50	5.46	4.95	5.20	1.90	1.89	1.87
75	4.62	3.99	4.30	1.79	1.58	1.68
100	5.28	4.45	4.87	1.26	1.57	1.42
Mean	4.85^{ns}	4.75		1.70^{ns}	1.69	
CV (%)	22.09			23.38		
Height (cm)						
0	31.75	30.58	31.16 a	35.16	37.25	36.20^{ns}
25	33.90	32.75	33.32 a	34.60	35.12	34.85
50	30.06	34.61	32.34 a	35.87	34.17	35.01
75	25.16	29.83	27.50 b	36.95	36.87	36.90
100	35.55	30.04	32.79 a	36.92	37.33	37.12
Mean	31.28^{ns}	31.56		35.90^{ns}	36.14	
CV (%)	7.96			11.10		

Means followed by the same lowercase letter within a row do not differ by the Scott–Knott test at the 5% significance level. *CV*, coefficient of variation; *ns*, not significant

Effect of *A. nodosum* extract on *M. javanica* egg hatch

A quadratic plateau model was fitted to experimental data with a coefficient of determination of 95% for Trial 1 and 73% for Trial 2. The minimum doses of *A. nodosum* extract to inhibit egg hatching was 29.5 g L^{-1} for Trial 1 and 30.3 g L^{-1} for Trial 2 (Fig. 1a, b), whose inhibition were greater than 92% in both experiments.

Effect of *A. nodosum* extract on induction of enzymes associated with plant resistance against *M. javanica* in soybean

This experiment was conducted once and enzymatic analyses were performed in duplicate. Significant interaction effects between treatment and evaluation time were observed on enzyme activity in soybean leaves. Peak POX and PPO activities occurred at 8 and 12 DAT in uninoculated plants treated by soil drenching (Fig. 2a, b). The effect of evaluation time was only significant for uninoculated plants treated by soil drenching, with the highest POX and PPO activities at 12 DAT and for inoculated plants treated by foliar spraying, with the highest POX activity at 12 DAT (Fig. 2a, b).

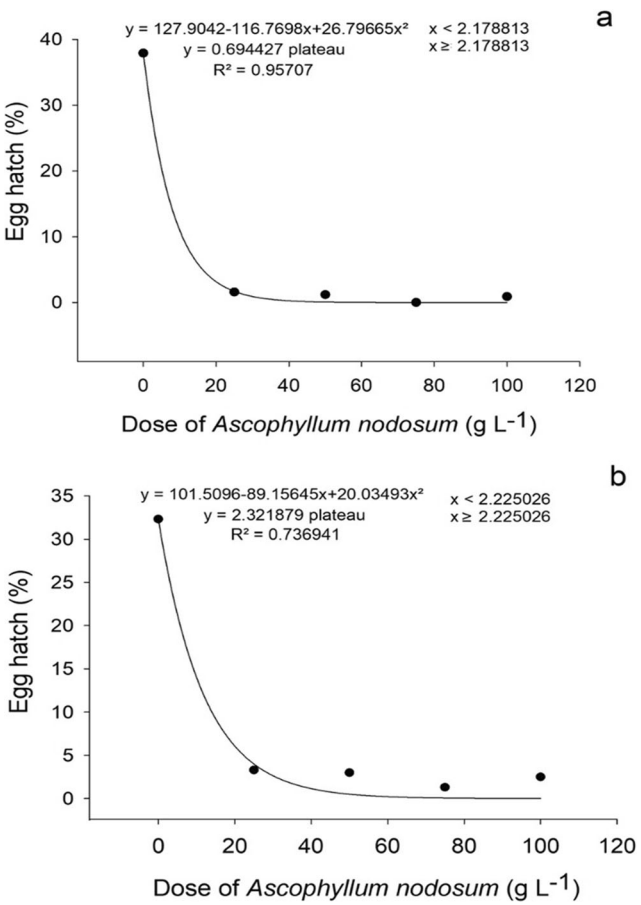

Fig. 1 *Meloidogyne javanica* egg hatching percentage in the presence of different doses of *A. nodosum* extract in **a** Trial 1 and **b** Trial 2

At 4 DAT, PAL activity was highest in inoculated plants treated by foliar spraying and, at 8 DAT, in uninoculated plants treated by soil drenching. The highest overall PAL activity was observed at 12 DAT in uninoculated plants treated by drenching (Fig. 3a). By analyzing differences between evaluation times, we observed that PAL activity was higher at 4 DAT in uninoculated sprayed plants and at 12 DAT in uninoculated drenched plants (Fig. 3a). GLU activity was detected only at 12 DAT in uninoculated drenched plants (Fig. 3b).

Discussion

Ascophyllum nodosum aqueous extract was effective in controlling *M. javanica* in soybean. Total nematode number, nematode population density, and gall index decreased significantly, in agreement with previous studies showing the

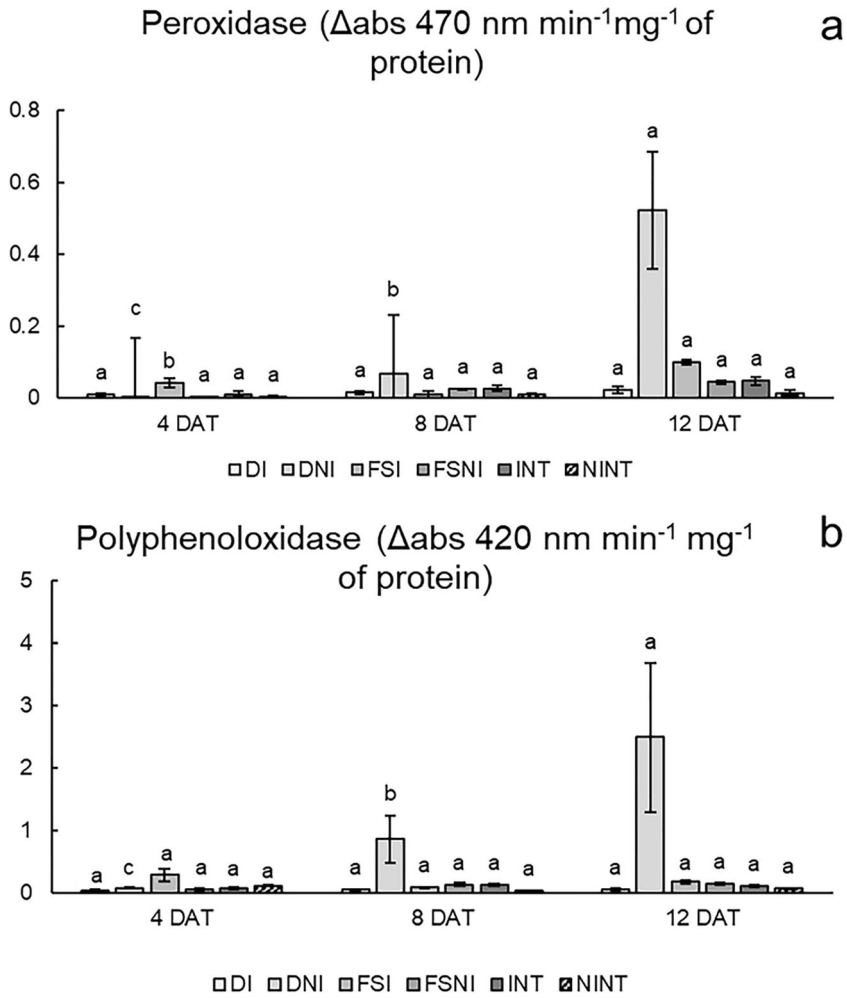

Fig. 2 **a** Peroxidase (POX) and **b** polyphenol oxidase (PPO) activities in leaves of soybean inoculated with *M. javanica* and treated or not with *A. nodosum* extract. DI, drenched inoculated plants; DNI, drenched noninoculated plants; FSI, foliar-sprayed inoculated plants; FSNI, foliar-sprayed noninoculated plants; INT, inoculated untreated control; NINT, noninoculated untreated control; DAT, days after treatment. Within each treatment, columns followed by the same letter do not differ from each other

efficacy of algal extracts in the management of different nematode species in plants (Whapham et al. 1994; Ara et al. 1996; Wu et al. 1997; Sultana et al. 2000; Noreen et al. 2002; Zaki et al. 2005). Extracts of *Sargassum tenerrimum*, *Melanothamnus afaqhusainii*, and *Padina tetrastromatica* caused more than 50% mortality in root-knot nematodes (Khan et al. 2015). Soil application of liquid fertilizers based on aqueous alkaline extracts of *A. nodosum* and *Ecklonia maxima* reduced *M. chitwoodi* number in tomato (Ngala et al. 2016). The number of *M. javanica* females in *A. thaliana* decreased after treatment with *A. nodosum* alkaline extract (Wu et al. 1998).

Ascophyllum nodosum extract contains betaines, cytokinins, auxins, abscisic acid, gibberellins, and alginates (Carvalho et al. 2013). Its nematicidal effects has been mainly attributed to betaines (Wu et al. 1997). These molecules stimulate the production of chlorophyll in leaves (Genard et al. 1991) and protect cells, proteins, and enzymes against stress-induced damage (Wu et al. 1997). Furthermore, betaines are potent precursors of formaldehyde, which is associated with induction of disease resistance in plants (Manninger et al. 1992). When applied at low concentrations, betaines can increase plant resistance to fungal attack (Tyihak et al. 1988; Kraska and Schõnbeck 1992; Manninger et al. 1992). In the current study, it is possible that these compounds caused a similar effect against nematodes, resulting in reduced *M. javanica* levels in soybean roots.

The inhibitory effect of algal extract on nematode egg hatch was likely influenced by direct contact between extract and eggs. In vitro analysis showed that hatching was reduced by more than 95%, even at the lowest doses tested. *S. tenerrimum*, *P. tetrastromatica*, and *M. afaqhusainii* extracts were shown to decrease *M. javanica* egg hatching by 96% (Khan et al. 2015). *Meloidogyne chitwoodi* egg hatching decreased in the presence of 100 and 50% *A. nodosum* solutions (Ngala et al. 2016).

A number of enzymes, including lipase, proteinase, and chitinase, are involved in the hatching process of nematodes (Perry 1997). Some compounds in *A. nodosum* extract might have interrupted enzyme activities that are correlated with hatching and eggshell flexibility (Perry et al. 1992), a property required for successful hatching in *Meloidogyne* spp. Extracts of *Enteromorpha (Ulva) flexuosa*, *Dilsea carnosa*, *Codium fragile*, *Cystoseira myrica*, *Sargassum muticum*, and

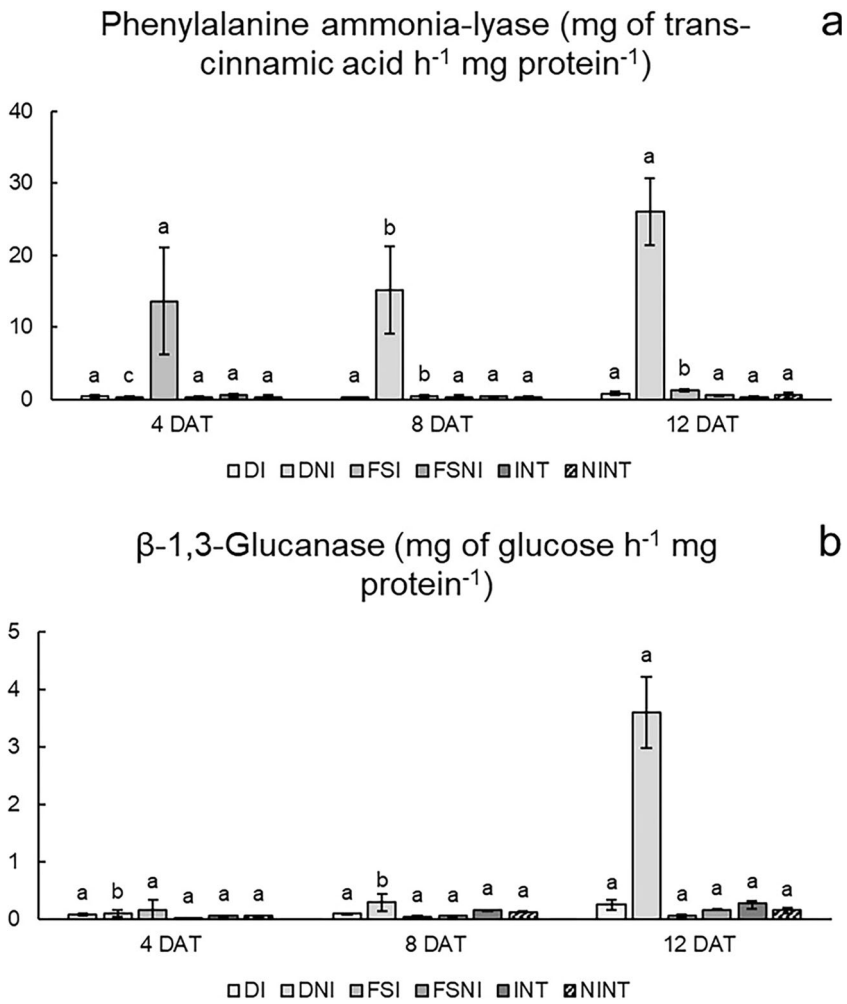

Fig. 3 **a** Phenylalanine ammonia-lyase (PAL) and **b** β-1,3-glucanase (GLU) activities in leaves of soybean inoculated with *M. javanica* and treated or not with *A. nodosum* extract. DI, drenched inoculated plants; DNI, drenched noninoculated plants; FSI, foliar-sprayed inoculated plants; FSNI, foliar-sprayed noninoculated plants; INT, inoculated untreated control; NINT, noninoculated untreated control; DAT, days after treatment. Within each treatment, columns followed by the same letter do not differ from each other

Laurencia nidifica reduced *M. incognita* egg hatching in a dose-dependent manner, confirming that brown algae produce secondary metabolites capable of inhibiting nematode egg hatching (El-Deen and Issa 2016).

It is important to note the effects of algal extract on nematode population might also have been influenced by algal mineral content. A previous study showed that nitrogen application to maize led to a reduction in *M. incognita* population, gall index, and egg mass index (Xavier 2020). Zhao et al. (2016) found that potassium fertilization of tomato seedlings reduced *M. incognita* infection. In another study, fertilizers containing calcium phosphonate, magnesium phosphonate, and potassium phosphonate increased the mortality of J2 and decreased hatching of *M. javanica* and *M. incognita* eggs in the in vivo (Habash and Al-Banna 2011).

Nutrients such as copper and zinc have been reported to have direct or indirect action on root-knot nematodes (Rumiani et al. 2016). Zinc was effective in controlling nematodes, reducing the number of *M. incognita* galls and eggs in tomato (Couto et al. 2016). Control of *P. brachyurus* in soybean was achieved by using manganese alone or in combination with other minerals (Conduta et al. 2020). A fertilizer high in iron reduced the number of *M. arenaria* galls, although no significant effects were found on nematode reproduction (Kokalis-Burelle et al. 2010).

Fertilizers increase the level of resistance or tolerance of plants to pathogens and pests because they increase plant access to nutrients. Strong, healthy plants are more capable of compensating for the loss of photosynthesis or damage caused by pathogens, including nematodes (Khan et al. 2012; Santana-Gomes et al. 2013). Studies have shown that plant mineral nutrition is an interesting nematode control strategy, as it favors plant development, thereby decreasing susceptibility to such parasites (Silva et al. 2006), and causes nematode death (Ketabchi et al. 2016). Further studies are needed to identify the effects of algal nutrients on nematodes.

High doses of algal extract applied by soil drenching increased soybean height. These effects might have been due to the presence of cytokinins in *A. nodosum* extract, which are known for their ability to regulate plant growth and stimulate cell division (Oliveira et al. 2011). Tomato plants treated with *A. nodosum* via soil application had superior growth

compared with untreated plants (Radwan et al. 2012). Bioactive substances extracted from *A. nodosum* probably promoted shoot development as well as root and foliar growth, contributing to overall plant growth (Fan et al. 2011). The growth-promoting effects of the extract can also be attributed to nutrient supply. Of note, potassium fertilization increases plant height (Lana et al. 2002), and nitrogen fertilization (up to 40 kg ha^{-1}) promotes shoot growth in soybean (Parente et al. 2015).

Ascophyllum nodosum extract partially enhanced shoot fresh and dry weights. The effect of algal extracts on shoot weight depends on their composition and concentration and can be either positive or negative (Costa et al. 2014). It is likely that, with the doses used in the present study, the algal extract was not able to exert physiological effects at its full potential.

Algae contain several carbohydrate molecules organized mainly in the form of oligosaccharides, including oligogalacturonides and some polysaccharides. Oligosaccharides are known to act as resistance elicitors and signal transduction molecules in plants (Vidhyasekaran 1997; Walters et al. 2005). Thus, the effective control of *M. javanica* obtained by treatment of soybean with *A. nodosum* extract may be explained by the elicitor effect of oligosaccharides present in the algal extract. Oligogalacturonide is systemically mobile and can induce the expression of various defense-related proteins and proteinase inhibitors in vivo (Ryan and Farmer 1991).

Resistance enzymes are involved in the synthesis of phytoalexins, phenols, and lignins, and their modulation can impair nematode feeding and mobility in plants (Seenivasan 2011; Vaganan et al. 2014; Sankar et al. 2017). Lignin, for instance, is composed of aromatic phenolic metabolites that are actively deposited on the cell wall during an infection process to act as a mechanism of resistance to pathogen penetration (Boudet et al. 1995).

The enzyme analysis experiment was performed only once. The potential indirect action of *A. nodosum* extract against nematodes was evidenced by the increased activity of resistance-related enzymes (POX, PPO, PAL, and GLU). Inoculated plants treated by foliar spraying showed peak enzyme activities at 4 DAT, whereas uninoculated drenched plants showed peak enzyme activities at 8 and 12 DAT. An increase in the activity of the enzymes PPO, POX, and PAL was observed in banana roots inoculated with *Radopholus similis* (Sankar et al. 2017). Similarly, sugarcane inoculated with *M. incognita* showed an increase in POX and GLU activities at 14 days after inoculation (Guimarães et al. 2010).

POX can act directly on plant defense against pathogens and plays an important role in signaling pathways associated with essential physiological processes, such as ethylene biosynthesis, indoleacetic acid decarboxylation, and lignification and suberization of cell walls (Pinto et al. 2011). As a result of POX activity, plant cell walls become more resistant to compression and degradation by hydrolytic enzymes from phytopathogens, such as plant-parasitic nematodes (Wuyts et al. 2006; Almagro et al. 2009; Barros-Rios et al. 2015; Bento-Silva et al. 2018). Thus, in the present study increased POX activity might have stimulated cell wall lignification, affecting nematode penetration and nutrition. Similar results to those observed here for *A. nodosum* extract were reported for tomato inoculated with *M. incognita*. Tomato plants treated with *Corallina mediterranea*, *Corallina officinalis*, and *Ulva fasciata* algal extracts, resulting in higher POX and PPO activities (Ghareeb et al. 2019). POX activity was enhanced in resistant and tolerant mutants of banana inoculated with *Pratylenchus coffeae* (Devi et al. 2007).

PPO catalyzes the *o*-hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones, which are highly toxic to microorganisms (Mishra and Gautam 2016). There was an increase in PPO activity in plants inoculated with *M. incognita*, indicating the possibility that this enzyme is involved in the mechanism of resistance to nematodes (Mazzafera et al. 1989). Similar results were observed by Shimizu (2004), who found that PPO activity in soybean plants increased after inoculation with *Heterodera glycines* and *M. javanica*. PPO and POX contribute to lignification by oxidizing a variety of phenolic compounds (Marjamaa et al. 2009; Živković et al. 2010).

PAL is a secondary metabolism enzyme and is crucial in reactions with phenolic compounds. It catalyzes the first reaction in the phenylpropanoid pathway, converting L-phenylalanine to cinnamic acid, affording phytoalexins and lignin precursors (monolignols), strengthening plant cell walls (Nicholson and Hammerschmidt 1992). Application of *cis*-jasmone induced the production of defense compounds and increased PAL activity in soybean; the phytoalexin was associated with soybean resistance to *M. javanica* (Janegitz et al. 2012), as also observed in the present study, in which there was an increase in PAL activity in inoculated and treated plants. An increase in PAL activity after nematode inoculation has been observed in potato, tomato, and soybean (Giebel 1974; Edens et al. 1995).

Joint action of POX, PPO, and PAL can promote structural changes that contribute to cell wall lignification, preventing the access of degrading enzymes produced by nematodes to plant cell wall polysaccharides (Grabber et al. 1998; Hiraga et al. 2001; Holbein et al. 2016). These effects are particularly important as a response to *Meloidogyne* spp., because root-knot nematodes must first penetrate the host root to gain access to food. Penetration occurs at the root apex, where cells are more fragile and less lignified (Karssen and Moens 2006). Following penetration, the nematode must migrate through the cortex to reach cells of the vascular cylinder (Karssen and Moens 2006). Establishment of nematode feeding sites depends on cell hypertrophy and the passage of nutrients from

nurse cells to giant cells (Ferraz and Brown 2016). Therefore, lignin accumulation can directly compromise nematode feeding, as it strengthens the cell wall and acts as a physical barrier. In a histological study of the root system of banana accessions resistant to *P. coffeae*, a high number of phenolic compounds and lignified cells were observed, which prevented nematodes from entering the cortex, preserving cell integrity. In the cortical parenchyma of susceptible cultivars, the authors observed a large number of cavities and deformed cells resulting from nematode penetration (Devi et al. 2009).

Soil application of acibenzolar-*S*-methyl reduced *M. incognita* gall number, egg mass, and egg density in tomato (Melillo et al. 2014). Such a reduction was associated with impaired nematode nutrition, as giant cells were found to have a small diameter. Lignin accumulation was observed in giant cells at 2, 3, and 7 days after inoculation. According to the authors, lignification might have increased the rigidity of the cell wall, negatively influencing the development of feeding sites (Melillo et al. 2014).

GLU is a pathogenesis-related protein and degrader of β-1,3-glucan, a cellular component of some pathogenic fungi and nematodes (Punja and Zhang 1993). β-1,3-Glucan degradation results in the release of oligosaccharides that elicit plant defense responses (Wu and Bradford 2003). GLU expression promotes the accumulation of phytoalexins (Edreva 2004), important secondary metabolites produced in response to biotic and abiotic stresses. Phytoalexins possess antimicrobial properties against fungi, bacteria, nematodes, and other pathogens (Ahuja et al. 2012). Activation of such responses in plants can occur through exposure to elicitors or pathogens (Hei et al. 2012). In the present study, both *A. nodosum* extract and *M. javanica* might have acted as elicitors of plant defense enzymes. Studies carried out on vines parasitized with *M. javanica* and *M. incognita* observed that GLU activity increased at 28 days after inoculation (Owen et al. 1998). Palanisamy and Kathiresan (2012) reported increased GLU activity in sugarcane inoculated with *P. zeae*, attributed to an increase as a probable defense reaction of the plant against the nematode.

Application of *A. nodosum* extract by soil drenching or foliar spraying enhanced enzymatic activity in inoculated and uninoculated soybean plants compared with controls, showing that the seaweed extract as well as the nutrients present in the product, can stimulate for increased activity of defense enzymes. These effects have been observed in other plant–pathosystems subjected to resistance induction as well as in plants expressing resistance genes. Induction of pathogen resistance is an attractive strategy that can activate multiple defense responses, providing protection against various pathogens (Anderson et al. 2006).

The results show that *A. nodosum* extract has the potential to control *M. javanica*, stimulate defense enzyme activity, and promote growth in soybean. Further studies are needed to validate the use of *A. nodosum* extract for the control of nematodes in the field and assess its economic viability. This control strategy can potentially reduce environmental impacts associated with synthetic products.

Conclusion

Ascophyllum nodosum extract was effective in reducing *M. javanica* populations in soybean roots. Extract application increased POX, PPO, PAL, and GLU activities at 12 DAT in uninoculated plants treated by soil drenching. *A. nodosum* extract has the potential to control *M. javanica* and induce nematode resistance in soybean.

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Declarations

Conflict of interest The authors declare no competing interests.

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