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Compatibility Between Entomopathogenic Nematodes and Different Commercial Seaweed Extracts

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Abstract

Biological control agents, specifically entomopathogenic nematodes (EPNs), appear as promising alternatives for insect control when considering the toxic damages caused by the excessive use of synthetic insecticides in agriculture. In many cases, EPNs are applied in combination with other agricultural inputs, which may include seaweed extracts. The question raised is whether these extracts are compatible or not with EPNs. The main objective of this research was to evaluate the compatibility between EPNs and different seaweed extracts. The study was run as a three single-factor experiment, using a completely randomized design. Three commercial seaweed extracts (Acadian®, SeamelPure® e SuperFifty®), manufactured using different hydrolysis processes, were evaluated using three nematode isolates. Each treatment was replicated five times. Each repetition consisted of a test tube with 2 mL of a suspension containing 1500 infective juveniles (IJs) and 15 mL of the diluted product or water (control). The parameters assessed included viability of EPNs, infectivity over *Tenebrio molitor* larvae and IJs reproductive potential after the contact with seaweed extracts. All seaweed extracts significantly affected the viability, infectivity and reproduction of all EPNs isolates tested. Nevertheless, Seamel PURE®, which is manufactured though an enzymatic instead of a highly aggressive alkaline hydrolysis process, was the only extract considered slightly harmful regarding the infectivity of all isolates tested. EPNs reproduction following the contact with each seaweed extract tested was reduced by 100% across all tested isolates. These findings indicate that under the tested conditions, seaweed extracts based on chemical hydrolysis are greatly incompatible with the evaluated nematode isolates.

Keywords: Biological control; *Heterorhabditis* spp.; *Steinernema* spp.; *Ascophyllum nodosum*; entomopathogenic

Introduction

With the growing global demand for food, a significant increase in the use of synthetic pesticides for the control of insect pests has been observed since the 1950's as this method has become one of the primary strategies for managing agricultural pests (Soria *et al*. 2017). However, the indiscriminate use of these products has led to several issues, ranging from environmental damage, risks to human health, harm to non-target organisms, to the development of resistance in many pest species (Qie *et al*. 2020).

As a consequence of the negative effects associated with the excessive use of synthetic insecticides, new alternative control methods have been sought. In recent years, biological control products have emerged as promising alternatives for increasing plants' self-defense, disease and insect control. Examples may include microbial control using entomopathogenic nematodes (EPNs) and seaweed extracts to increase plants' defense against biotic and abiotic stresses.

The two main genera of EPNs, *Steinernema* and *Heterorhabditis*, are effective against a wide range of economically significant insect pests (Laznik and Trdan 2014). These agents exhibit strong potential as biological control agents due to their ability to form symbiotic associations with bacteria. These bacteria are responsible to kill the host insect and produce substances that benefit the nematode, while the nematode acts as a disperser of the bacteria in the environment (Lacey *et al*. 2015). EPNs can be as effective as synthetic insecticides when applied under favorable conditions, such as high humidity, optimal

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temperature, and low ultraviolet radiation. In general, they are also compatible with other agricultural products, allowing for their simultaneous or sequential application with synthetic pesticides to manage various insect pests (Laznik and Trdan 2017).

Seaweeds, on the other hand, act on plants by enhancing the biosynthesis of specialized metabolities, which increase the plants' resistance to insects and pathogens. Moreover, these substances can also elicit the production of a variety of bioactive compounds that help plants cope with adverse environmental conditions (abiotic stresses) (Ducatti *et al*. 2022) while helping plants to increase nutrient utilization and enhance plant vigor (Arioli *et al.* 2024). Among these compounds, polyanionic polysaccharides uniquely found in seaweed – such as alginates, fucoidans, laminarins, ulvans, carrageenans and agar – stand out as the primary elicitors for terrestrial plants (Shukla *et al*. 2021).

Nevertheless, one of the key aspects concerning farmers and researchers about the application of different biological control agents is their compatibility. When two control agents act independently on a target host and the toxicity of one is not affected by the other, their combined effects may be enhanced (Robertson *et al*. 2007).

Seaweed use in agriculture more than doubled from 2006 to 2021 and projections show an increase in its market value ranging from 6 to 10% annually until 2032-2035 (Yasmeen *et al.* 2025). However, due to the diverse methods used in manufacturing seaweed extracts, which include, but are not limited to, acid, alkaline and enzymatic hydrolysis (Shukla *et al*. 2019), little is known about their compatibility with EPNs.

Therefore, the objective of this study was to evaluate whether there is compatibility between EPNs and different commercial seaweed extracts manufactured using different types of hydrolysis.

Materials and Methods

EPNs were collected from random regions of Chapecó and Xaxim, cities located in Western Santa Catarina – Brazil and multiplied in *Tenebrio molitor* L. larvae (Coleoptera: Tenebrionidae) reared according to the method described by Potrich *et al*. (2007). Multiplied EPNs were retrieved following the methodology proposed by Molina and López (2001).

Three single-factor experiments were conducted in a completely randomized design using an adapted protocol described in the "International Organization for Biological and Integrated Control – West Palaearctic Regional Section" (IOBC/WPRS) and proposed by Vainio (1992). The experiments involved the use of three commercial seaweed-based products (SuperFifty®, Acadian® and SeamelPure®) and three EPNs isolates (Table 1). Each treatment was replicated five times.

For each product, one liter of solution was prepared by diluting 5 mL of the commercial product in one liter of distilled water, simulating the proportional amount used per ha. From each of these solutions, 15 mL aliquots were taken and placed into test tubes containing 2 mL of a suspended solution composed of 1500 infective juveniles (IJs). Therefore, each tube contained a total volume of 17 mL (15 mL of product solution + 2 mL of EPNs suspension). The control treatment consisted of test tubes containing 15 mL of distilled water + 2 mL of IJs suspension (1500 IJs). Tubes were sealed and kept in the dark in a B.O.D. (Biochemical Oxygen Demand) chamber under 24 ± 1°C and 70 ± 10% relative humidity for 48 h.

Thereafter, the viability of the EPNs was assessed. Tubes were homogenized by shaking, and 0.1 mL aliquots of the suspension were placed into 10 wells of an ELISA (Enzyme-Linked Immunosorbent Assay) plate for observation under a stereomicroscope. The number of live and dead IJs in each aliquot was recorded. Individuals that remained immobile with fully extended bodies were considered dead.

After viability was confirmed, EPNs exposed to the respective treatments underwent infectivity assessment. Suspensions were washed three times with distilled water, each cycle involving a 30-min decantation period in a B.O.D. chamber, followed by removal of approximately 8.5 mL of the supernatant and replacement with an equal volume of distilled water. After the final wash, suspensions were homogenized by agitation, and 2 mL aliquots were transferred to Petri dishes containing two sheets of filter paper and ten live *T. molitor* larvae. Petri dishes were incubated under the same B.O.D. conditions as in the previous experiment for 48 h. Larvae that died during this period were transferred to fresh Petri dishes with filter paper and incubated for an additional five days under the same conditions to confirm infection.

To assess IJs reproduction, larvae killed by the nematodes were transferred to White traps and maintained under the same B.O.D. conditions. Emerged IJs were quantified under a stereomicroscope for a period of 15 days.

Normality and homoscedasticity assumptions were tested using the Shapiro-Wilk and Bartlett tests, respectively. Subsequently, as both parameters indicated the presence of non-parametric data, the viability and infectivity data of the JIs were subjected to Kruskal-Wallis ($P < 0.05$) followed by the post-hoc Mann-Whitney ($P < 0.05$) mean comparison test (R Core Team 2022).

Nematode mortality values were corrected using Abbott's formula (1925):

$$Mco\% = \left(\frac{Mt\% - Mc\%}{100 - Mc\%}\right) \text{x } 100$$

Where: *Mco%* refers to the corrected mortality; *Mt%* corresponds to the observed mortality and *Mc%* represents the mortality observed for the control treatment.

Table 1: Entomopathogenic nematode (EPNs) isolates and their viability

EPNs isolate	Species	Viability (%)*
UENP	*Heterorhabditis amazonensis*	96
APII	*Oscheius tipulae*	93
UFFS	*Oscheius tipulae*	94

*Viability was checked prior to starting the experiment

Table 2: Normality and homoscedasticity *P values* for the viability and infectivity of three entomopathogenic nematodes (EPNs) isolates tested in combination with different commercial seaweed extracts

EPNs isolate	Viability		Infectivity	
	Normality (Shapiro-Wilk)	Homoscedasticity (Barlett)	Normality (Shapiro-Wilk)	Homoscedasticity (Barlett)
UENP	0.0014	0.0005	0.0003	0.3681
APII	0.0003	< 0.0001	0.0003	0.0904
UFFS	0.0017	0.0007	0.0082	0.0159

*Numbers in bold are significant and represent data with abnormal distribution and not homogeneous

Table 3: Kruskal-Wallis tables for three experiments using entomopathogenic nematodes (EPNs) isolates tested in combination with different commercial seaweed extracts

EPNs isolate	Viability			Infectivity		
	ChiSquare	DF	*P* > ChiSq	ChiSquare	DF	*P* > ChiSq
UENP	16.0743	3	0.0011	11.8175	3	0.0080
APII	12.9543	3	0.0047	13.6254	3	0.0035
UFFS	15.7543	3	0.0013	13.3659	3	0.0039

*Numbers in bold are significant and indicate the existence of significant differences between the means of each treatment. DF: Degrees of freedom

The reduction in infectivity was determined by the percentage of dead *T. molitor* larvae and was calculated using Vainio's (1992) formula:

$$Rinf\% = \left(1 - \frac{It\%}{Ic\%}\right) x\ 100$$

Where: *Rinf%* refers to the reduced infectivity; *It%* corresponds to the observed infectivity; and *Ic%* represents the infectivity observed for the control treatment. The reduction in infectivity was used to classify the products following the guidelines of IOBC/WPRS: Harmless (< 30%), Slightly harmful (between 30% and 79%), Moderately harmful (between 80 and 99) and Harmful (> 99) to EPNs (Peters and Poullot 2004).

The reduction in nematode reproduction was assessed by counting the IJs that emerged from *T. molitor* larvae in the white traps and calculated using Vainio's (1992) formula:

$$Rfec\% = \left(1 - \frac{Ft\%}{Fc\%}\right) \text{x } 100$$

Where: *Rfec%* refers to the reduced reproduction; *Ft%* corresponds to the observed reproduction and *Fc%* represents the reproduction observed for the control treatment.

Results

The *P values* for the normality and homoscedasticity analyses referring to the viability and infectivity variables in the three experiments conducted with EPNs isolates and different seaweed extracts can be found on Table 2, while Table 3 brings the results for the non-parametric ANOVA (Kruskal-Wallis).

The results obtained from the interaction between the different seaweed extracts and EPNs were statistically significant (Table 4). For the APII isolate, exposure to Acadian®, SeamelPure® and SuperFifty® exhibited viability reductions exceeding 45%, with no statistical differences between treatments. A similar outcome was observed for the infectivity in this isolate, which was reduced by over 72%. Seamel Pure® showed the least reduction in viability and infectivity for this isolate. Nevertheless, the lowest corrected mortality was observed for the SuperFifty® treatment.

While a significant lethal effect on EPNs survival was observed with all product applications, the highest nematode mortality (Mco%) for isolates UENP (48.67%) and UFFS (72.34%) occurred in treatments with Acadian® and SuperFifty®, respectively. Controversially, for isolate APII, Seamel PURE® was the responsible for the highest mortality of EPNs (72.80%).

Compared to the control, *H. amazonensis* IJs (Isolate UENP) showed decreased viability rates of 70.81% and 72.17%, respectively, when exposed to Acadian® and SuperFifty®. Exposure to SeamelPure® resulted in lower sensitivity, with viability reduced by only 38.3%. These findings align with the UFFS isolate, where IJs also exhibited lower sensitivity to SeamelPure®, with viability reduced by about 2.83%.

The infectivity assays showed that all nematode isolates experienced a reduction in infection rates following exposure to seaweed extracts. Specifically, the ability of nematodes to kill *T. molitor* larvae was reduced by over 49%, classifying all treatments to some degree, as harmful to EPNs. Nonetheless, Acadian® showed the lowest reduced infectivity (Rinf%) for the UFFS isolate while it also

Table 4: Compatibility of UENP (*Heterorhabditis amazonensis*), UFFS (*Oscheius tipulae)* and APII (*Oscheius tipulae*) isolates after 48 h of exposure to different seaweed extracts

EPNs Isolate	Treatment	Viability (%)	Infectivity (%)	Mco%	Rinf%	Rfec%	IOBC/WPRS Classification*
UENP	Acadian®	20.35 ± 5.85 c	18.00 ± 10.9 b	48.67	80.43	100.0	Moderately Harmful
	SeamelPure®	52.86 ± 9.16 b	24.00 ± 11.4 b	28.85	73.91	100.0	Slightly Harmful
	SuperFifty®	18.99 ± 2.52 c	18.00 ± 8.36 b	46.91	80.43	100.0	Moderately Harmful
	Control	91.16 ± 0.61 a	92.00 ± 4.47 a	0.0	0.0	0.0	Harmless
	CV (%)	66.89	87.32				
UFFS	Acadian®	44.62 ± 15.0 b	44.00 ± 28.8 bc	35.93	48.84	100.0	Slightly Harmful
	SeamelPure®	85.69 ± 5.47 a	40.00 ± 15.8 b	01.41	53.49	100.0	Slightly Harmful
	SuperFifty®	31.30 ± 10.4 b	18.00 ± 8.36 c	72.34	79.07	100.0	Moderately Harmful
	Control	88.52 ± 0.92 a	86.00 ± 5.47 a	0.0	0.0	0.0	Harmless
	CV (%)	43.41	63.30				
APII	Acadian®	19.96 ± 9.63 b	06.00 ± 8.94 b	62.69	93.18	100.0	Moderately Harmful
	SeamelPure®	42.17 ± 28.9 b	24.00 ± 20.7 b	72.80	72.72	100.0	Slightly Harmful
	SuperFifty®	26.64 ± 2.89 b	14.00 ± 5.47 b	53.70	84.09	100.0	Moderately Harmful
	Control	90.42 ± 0.62 a	88.00 ± 10.9 a	0.0	0.0	0.0	Harmless
	CV (%)	70.44	106.84				

Means followed by the same letter do not differ statistically according to Mann-Whitney test ($P < 0.05$)
CV(%) = Coefficient of variation; Mean ± Standard Deviation; Mco% = Corrected mortality; Rinf% = Reduced infectivity; Rfec% = Reduced reproduction
*IOBC/WPRS classification used to classify the reduction in infectivity of *Tenebrio molitor* larvae by entomopathogenic nematodes after they have been exposed to the seaweed extracts (Rinf%). Harmless (< 30%), slightly harmful (between 30% and 79%), moderately harmful (between 80 and 99) and harmful (> 99) to EPNs (Peters and Poullot 2004)

presented the greatest Rinf% for isolates UENP and APII. None of the isolates exposed to the three products produced progeny in *T. molitor* larvae – unlike the control treatment, which did enable reproduction.

Discussion

In general, the effect of the three products on the survival of IJs varied according to the EPNs isolates, supporting the hypothesis that compatibility is not solely species-specific but also isolate-specific (Laznik and Trdan 2014).

Acadian® and SuperFifty® are seaweed extracts produced with the macro seaweed *Ascophyllum nodosum* through an alkaline hydrolysis (Shukla *et al*. 2019; Rasul *et al*. 2021). Since plants can only absorb and use water-soluble substances (Ducatti and Tironi 2024) hydrolysis is a process indispensable to produce seaweed extracts and turn insoluble sugars/compounds into soluble and absorbed molecules (Gaige *et al*. 2020).

Because *A. nodosum* is considered a perennial and thus more "rigid" species (Marbà *et al*. 2017), industries usually employ highly aggressive chemical hydrolysis with sodium hydroxide, potassium hydroxide, and/or potassium carbonate to rapidly solubilize its components (Shukla *et al*. 2019). One consequence of such extract is a product with a very high pH (pH > 8.5), which can be harmful to organisms at high concentrations (Nisa *et al*. 2021). Acidic hydrolysis of *A. nodosum* is usually not performed for products designed for agricultural use, as this hydrolysis converts alginates, the main elicitor component in *A. nodosum*, into insoluble alginic acid (Shukla *et al*. 2019; Abka-Khajouei *et al*. 2022) preventing it from being absorbed by plants (Ducatti and Tironi 2024).

For instance, Halebian *et al*. (1981) showed that well diluted KOH solutions (3%) could easily disrupt gram-negative bacteria cell walls, while Xiao *et al.* (2015) showed that pH increases (7.0 – 12.5) caused damages and disruption of cell structures of microorganisms present in sludge. For *A. nodosum*, as stated before, alkaline solutions and high temperatures are frequently used to disrupt cell walls and recover the intracellular soluble content that can be used by plants and other organisms (Zhang *et al.* 2024). There is a large likelihood that, because of the time that EPNs remained in contact with the diluted alkaline seaweed extracts, the presence of KOH could have caused some sort of cell damage to EPNs. It has been shown that alkaline solutions of KOH and NaOH can cause modifications in chitin solubility (component of the cuticle of nematodes) turning it to be water-soluble (Wang *et al.* 2025). This would lead to a loss of structural integrity and cause the death of EPNs (Phiri *et al.* 2013).

Conversely, SeamelPure® is produced with the macro seaweed *Solieria chordalis*. This seaweed is considered to have an annual life cycle (Burlot 2016), making it more malleable and suitable for enzymatic hydrolysis (Spain *et al*. 2024). This hydrolysis allows the solubilization of great amounts of bioactive molecules and yields a product with more acidic pH values and less harmful to EPNs.

The high reduction in viability observed for the interaction of EPNs with the three commercial seaweed extracts used in this experiment could be attributed to the secondary metabolites present in seaweed extracts – such as antioxidants, polyphenols, flavonoids, and other compounds – which may hinder nematode propagation and reduce their harmful effects to insects (Shawky *et al*. 2009). Seaweeds biosynthesize a wide range of specialized metabolites that mediate intra- and inter-specific ecological interactions. These extracts' nematicide activity may be attributed to both the chemical composition and concentration of these bioactive substances (Ibrahim *et al*. 2021).

Such example of specialized (bioactive) metabolites synthetized by seaweeds and present in these extracts are the

palmitic acid, dibutyl phthalate, tricarboxylic propane acid and methyl tetradecanoate, among others. These metabolities are responsible for inhibiting egg hatch, increasing larvae mortality and reducing root-not diseases caused by nematodes (Ghareeb *et al.* 2022).

The reduction in infectivity observed in Table 4 may be attributed to the presence of phenolic compounds and mineral salts that facilitate the penetration of seaweed compounds into nematodes, thereby enhancing its deleterious effects (El-Eslamboly *et al*. 2019). The chemical constituents present in these extracts may interfere with nematode infectivity by affecting lipid biosynthesis or metabolism, the primary energy source for these organisms, as observed in *H. amazonensis* RSC5, *H. amazonensis* JPM4, and *S. carpocapsae* in studies performed with *Galleria mellonella* larvae (Andaló *et al*. 2009).

Additional studies have also highlighted the antimicrobial activity of isolated compounds such as fatty acids, polysaccharides, phlorotannins, pigments, lectins, terpenoids, alkaloids and halogenated substances found in green, brown, and red algae (Pérez *et al*. 2016), which may also interfere in the infectivity of EPNs.

Conclusions

The three seaweed extracts caused high mortality in infective juveniles, prevented nematode reproduction, and affected their infectivity (as seen in Table 4). Nevertheless, seaweed extracts based on enzymatic hydrolysis were less harmful to EPNs and could be an alternative to farmers when using these biological agents in association with seaweed extracts. These findings indicate that under the tested conditions, the products, mainly those based on chemical hydrolysis, are greatly incompatible with the evaluated nematode isolates.

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Author Contributions

PEP and VSA conducted the experiments, identified the nematodes and helped on writing the manuscript. RDBD and SPT wrote and revised the paper. SMS and ALR conducted all the statistics and interpretation for the results while MAT supervised the works and designed the experiment.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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