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Nematode Extract and Acaciasides Use as Preventive Biomedicines Against Plant Diseases: Improved Earth-Environmental-Health-Research Science-Technology-Communication and May be Controlled 21st-Century Pandemic Diseases!

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Abstract
Traditional chemical control using chemical pesticides available for the last few decades is in a declining status internationally. Thus, there remains a need for developing effective biomedicines which would be cheap, non-phytotoxic, and non-pollutant. Animal-biomedicine, Nematode extract (NE) of the females of Meloidogyne incognita @ 0.14 mg/plant- and plant-biomedicines, acaciasides (A and B; two triterpenoid saponins) isolated from the funicles of Acacia auriculiformis @ 20 mg/plant, applied by foliar spray on okra plants (Abelmoschus esculentus L. Cv. Ankur-40) reduced M. incognita and yellow vein mosaic virus (YVMV) infection of the host plants and enhanced their growth. Treatment with NE followed by the application of acaciasides gave better control of root-knot and YVMV diseases and promoted higher growth of plants. The protein content of fruits has also been increased in both cases. Using double the dose of acaciasides alone, by foliar spray and soil drench gave no better results with respect to the control of nematodes and viral diseases and growth of the test plants. An analysis of protein, by electrophoresis and densitometer scanning of the test plants, showed that treatment with both the extracts resulted in the increased number of proteins in the roots. This indicates that both the extracts can induce the expression of some new pathogenesis-related (PR) proteins, which might have inhibited nematodes and YVMV infection like plant diseases. So, Nematode extract and acaciasides use as preventive potential biomedicines against plant diseases by improving agriculture, crop production, toxic-free earth-environmental-health and research, science-technology, and communication, and may be controlled 21st-century pandemic diseases by increasing natural immunity. And in the future synthetic production of biomedicine-NE will be cost-effective, easily maintainable supply-chains, accurate information, plans for ensuring sustainable developments, continuous supply of essential medicines by improving the biomedicines for the benefit of public health with the development of earth-environmental-health and research.

Keywords: Nematode-Extract-and-Acaciasides, Preventive-Biomedicines, Plant-and21st-Century-Pandemic-Diseases, Improved, Earth-Environmental-Health-Research-Science-Technology-Communication

Introduction

Traditional chemical control using chemical pesticides available for the last few decades is in a declining status internationally. Thus, there remains a need for developing effective pesticides which would be cheap, non-phytotoxic, and non-pollutant. Many plant substances provide effective nematicides which are easily biodegradable [1-7]. To overcome these situations, the safe alternative to the conventional method of control is to induce the natural defense response of the host plants against nematode attack and YVMV infection. Plants have a number of defense mechanisms against pathogens. A number of inducible defense responses are reported to have contributed to disease resistance in plants. Systemic resistance can be induced in different crop plants by non-pathogenic and pathogenic micro-organisms or their culture filtrates [8-14]. It is evident that salicylic acid (SA) is an endogenous signal for the activation of certain plant defense responses, including pathogenesis-related (PR) gene expression and the establishment of enhanced resistance [9,15-17]. Synthesis of

PR-proteins serves as a marker for disease resistance of tobacco plants [12]. Ethanolic extract of *Meloidogyne incognita* (Kofoid & White, Chitwood) females reduces root-knot disease and increases growth of plants [5-7,18]. The extract from the funicles of the plant *Acacia auriculiformis* A. Cunn. or its active principle acaciasides not only reduces *M. incognita* infection of plants and enhances their growth but also control mulberry foliar diseases [3-7,19].

The purpose of the present experiment is to see whether animal-biomedicine, nematode extract (NE) and plant-biomedicines, acaciasides (A & B) could reduce root-knot nematode infection of okra plants and also to find out if both the extracts can reduce a very common yellow vein mosaic virus (YVMV) disease of plants under naturally infected conditions by inducing defense response of host plants.

Materials and Methods

Preparation of Nematode Extract (NE)

M. incognita females were collected from the roots of okra plants grown in an experimental garden in the Department of Zoology, Visva-Bharati, Santiniketan-731235, West Bengal, India. They were washed with sterile distilled water, homogenized in a tissue homogenizer, and extracted with 90% of ethanol at room temperature (20 ± 2°C) for 5 days. The extract was allowed to evaporate at room temperature and the residue was kept over anhydrous calcium chloride for dehydration and stored at 4°C [5-7,18].

Isolation of Acaciasides

Air-dried and powdered funicles of A. *auriculiformi*s were successively extracted with petrol (60-80°C) and 90% ethanol. The ethanol extract, on removal of the solvent under reduced pressure, yielded a viscous dark brown mass. The extract was chromatographed on silica gel with petrol, petrol-chloroform (1:1), chloroform, chloroform-methanol (9:1, 7:3, 3:2, 1:1, and 2:3) as successive eluents. The chloroform methanol (7:3 and 3:2) eluates were then combined. The combined fraction was found to be composed mainly of two compounds which were separated by repeated preparative IIPLC employing Spherisorb S-IO-ODS reserved phase column with the solvent system methanol-water (7:3) at a flow rate of 4 ml/min and refractive index detector as white amorphous solids. These two solid compounds, designated as acaciaside A and B according to their increasing order of polarity, were found to be triterpenoid saponins by Liebermann-Burchard, molisch and forth tests [19-22].

Foliar Disease (YVMV)

The naturally infected foliar-YVMV disease was observed in the test plants during the experiment and identified according to their characteristic symptoms by the expert concerned. Diseased leaves were counted in each group [4, 7, 21-25].

Inoculation and Treatments

Aseptically germinated seeds of *Abelmoschus esculentus* (L.) Moench Cv. Ankur-40 was sown at the rate of one seed/pot (32 cm diam.) containing a mixture of clay soil and composted manure (2:1 v/v). The soil-filled pots were treated with boiling water 5 (five) times. The pots were divided into the following groups; each numbering 10: (i) uninoculated untreated, (ii) inoculated untreated, (iii) uninoculated NE-treated, (iv) NE-treated before inoculation and (v) acaciasides-treated after inoculation, (vi) NE-treated after inoculation, (vii) acaciasides-treated after inoculation and (viii) acaciasides-treated after inoculation by foliar spray and soil drench. Except for the last group, all other treatments were followed by foliar spray. The experiment was conducted outdoors at an ambient atmospheric temperature (27 ± 2°C) and relative humidity (75 ± 5%) [1-7].

Plants were inoculated at the 6-leaf stage (Day-20) with *M. incognita* (J2) @ 3425±75 larvae/plant. Dehydrated NE was mixed with sterile distilled water @ 0.06 mg/ml and sprayed on leaves of test plants at 0.14 mg/plant. Acaciasides were dissolved in sterile distilled water @ 2 mg/ml and sprayed on plants at 20 mg/plant. In the last treatment group, each plant received 40 mg of acaciasides; 20 mg from foliar spray, and 20 mg from soil drench. Solution of acaciasides was sprayed on leaves in 5 installments spanning 2.5hr. interval. Pre- and post-inoculation treatments were given 3-days before and 3-days after inoculation with nematodes and the plants were regularly watered in the morning and evening. During spraying, the soil surface underneath each plant was covered with a polythene sheet. Plants in both uninoculated untreated and inoculated untreated groups received a spray of an equal amount of distilled water [1-7].

Harvesting

All the plants were uprooted 85 days after the sowing of germinated seeds and the following parameters in all the 8 groups of plants were recorded: biomass of shoot and root, no. of leaves/plant, no. of virus-infected leaves/plant, no. of root galls/plant, nematode population/2g root and 200g soil, root- and fruit-protein content percent. Nematodes were extracted from soil and root by the modified Baermann method. Roots were finely torn into pieces by a blender before nematode extraction [5-7]. Three samples of fruit and root pieces were taken at random from each treatment and the total protein fraction in each sample was estimated by the Folin-Phenol method [26-27]. The experiment was repeated thrice with similar results. Data from the last experiment were presented (Table 1) and the data were analyzed by the analysis of variance (ANOVA).

Toxicity Test

Nematode extract has no direct toxic effect on nematode [5-6] but acaciasides have been found to be highly toxic to the nematode [18].

Densitometer Scanning

Root protein separation was carried out essentially by the method of Laemmli (1970) with the modifications as suggested by the LKB Instructional Manual (1986). A 10% separating gel and 5% stacking gel were used. The bands were scanned with a recording electrophoretic scanner (Biomidi, 96-300 densitometers). Observation was recorded from the densitometer curve [22, 28].

Results

Data were analysed by ANOVA and results are given in Table 1. Treatment with NE before inoculation and treatment with acaciasides reduced nematode infestation in terms of root gall number and nematode population in root to a minimum among all the treatment groups (Table 1). While acaciasides alone reduced YVMV infection of leaves to a minimum, NE and acaciasides together came next in order of efficacy among all the treatments (Table 1). Nematode population in rhizospheric soil was maximum with the group treated with NE + acaciasides and minimum with the untreated group (Table 1). Plant growth in terms of biomass of shoot and number of leaves was maximum with uninoculated and NE-treated groups followed by the groups which were treated with NE + acaciasides and which were uninoculated and untreated (Table 1).

Table 1: Effects of nematode extract and acaciasides on the biomass, no. of leaves/plant, no. of virus infected leaves/plant, no. of root galls, nematode population in soil and root, root and fruit protein content (%), inoculated with *M. incognita* J2 and naturally infected with YVMV.

Treatment Groups	Average Biomass (g)		Average Number of Leaves/ Plant	Average Number of Virus-Infected Leaves/ Plant	Average Number of Root-Galls	Average Nematode Population		Average Protein content (%)		Average Number of Total Protein
	Shoot	Root				Soil (200g)	Root (2g)	Root	Fruit	
1.Uninoculated Untreated	126.25b ±4.34	16.12d ±0.23	11.34ab ±0.33	6.20a ±0.16 (78.00%)	Nil	Nil	Nil	1.14h ±0.06	1.87c ±0.26	11
2. Inoculated Untreated	118.23c ±3.22	27.72b ±0.09	9.64b ±0.21	6.40a ±0.18 (84.00%)	348.80a ±15.30	30.20e ±0.14	708.62a ±8.35	2.89a ±0.09	1.12d ±0.03	18
3. Uninoculated NE-Pretreated (Foliar)	157.303 ±4.26	18.02cd ±0.67	12.87a ±0.27	5.80a ±0.05 (71.00%)	Nil	Nil	Nil	1.43d ±0.05	2.34a ±0.05	24
4. NE-Pretreated Inoculated	107.48d ±3.23	14.12d ±0.18	11.38ab ±0.46	3 90ab ±0.01 (50.00)	50.86d ±1.14	970.88b ±5.37	22.68c ±0.72	1.17g ±0.02	2.14b ±0.03	23
5. NE-Pretreated Acaciasides Posttreated (Foliar)	107.12d ±2.11	49.12a ±1.76	12.77a ±0.35	2.10b ±0.04 (25.00%)	23.22e ±0.72	1320.38a ±16.24	18.98f ±0.16	1.79c ±0.07	2.36a ±0.04	20
6. Inoculated NE-Post treated	105.13d ±2.96	18.20cd ±0.32	11.81a ±0.46	5.00ab ±0.04 (53.34%)	51.25c ±1.25	474.68c ±4.65	42.02d ±0.46	1.34e ±0.04	1.42d ±0.05	16
7. Acaciasides Posttreated	108.3 ld ±2.17	20.98c ±0.72	9.63b ±0.27	2.50b ±0.02 (8.25%)	59.1 lb ±0.48	940.0 lb ±4.83	202.12b ±1.48	1.93b ±0.07	1.32c ±0.03	15
8. Acaciasides by Foliar Spray and Soil Drench	103.30d ±1.13	15.62cd ±0.37	10.67ab ±0.30	1.40b ±0.01 (7.15%)	24.98c ±0.78	160.12b ±2.24	164.59c ±3.73	1.2 lf ±0.03	2.19b ±0.03	19

a, b, c ... : Different small letters in a column indicate significant difference (P≤0.05) by ANOVA. Except the last group all the treatments are by foliar spray.

Protein content percent in fruits was maximum in the groups treated with NE + acaciasides and also in the group which were uninoculated but NE- treated (Table 1) and root protein content was maximum in inoculated untreated group. In general, all the treatments reduced nematodes and virus (YVMV) infection of the test plant significantly (Table 1). Treatment with acaciasides by both foliar spray and soil drench gave a very significant measure of control of both nematodes and YVMV infection (Table 1).

An analysis of root protein of all groups by electrophoresis and densitometer scanning of all the test plants show that both the treatments (NE and acaciasides) resulted in increased number of proteins in the roots; the lowest number of protein is 11 in uninoculated untreated roots and the highest number of protein is 24 in uninoculated NE- treated foliar roots (Table 1).

Discussion

The present experiment once again confirms our previous observation that besides reducing root-knot disease, both the biomedicines; acaciasides (A & B) also suppress foliar diseases caused by virus, fungus, and insects in mulberry plants [4], and nematode extract (NE) could significantly reduce *M. incognita* infestation of plants and that pre inoculation treatment was more effective than post inoculation one [5-7], and it is interesting to note that the animal-biomedicines, NE could also ameliorate the yellow vein mosaic virus (YVMV) infection of the okra plants. Treatment with the both plant- and animal-biomedicines; acaciasides and nematode extract leaves no toxic residues on test plants [3-9,21-25].

It is possible that the both plant- and animal-biomedicines; nematode extract and acaciasides systematically could active natural defense mechanisms in the test plants. *M.incognita* is known to share common antigens with its host plants [29]. It appeared that during natural infection with the nematode, host plants showed minimal defense responses to the nematodes because of this antigenic similarity. In this experiment, NE containing various antigens and acaciasides may induce defense responses involving a number of pathogenesis-related (PR) proteins in which the nematodes and YVMV fail to tolerate. Root invasion by the cyst nematode produced systemic accumulation of new proteins in the leaves of potato plants [30]. Lectins accumulated in gall-regions of roots of lady's finger plants infected with *M. incognita* [31]. Root-knot nematodes alter patterns of plant gene expression within cells destined to become the feeding site for them [32]. Systemic defense response is induced locally by pathogen or pest attack and is mediated by salicylic acid which induces expression of pathogenesis-related genes [33]. SA enhances resistance against *M. incognita* by inducing expression and accumulation of pathogenesis-related–I protein (14 kD, PR-I) in sprayed plant roots and leaves [17]. Osbourn (1996) reported that saponins also provide defense to the plants against pathogenic fungi [34]. Thiamine, polyacrylic acid, 2,6-dichloroisonicotinic acid are also involved in PR gene expression and enhance resistance through a different pathway [35].

An analysis of root proteins by gel electrophoresis and densitometer scanning, the result indicates that the lowest number of proteins in the roots of the test plant was found in uninoculated untreated plants (No. 11). In the inoculated untreated plant, number of protein increase (No. 18). This shows that infection of *M. incognita* and YVMV result in the expression of some new proteins in the test plants [22-25]. However, the plants treated with the NE showed the highest number of proteins 23 and 24. In this present treatment was given before inoculation with nematodes or without any inoculation. This shows that NE serves as a stimulus for the expression of many proteins particularly the defense-related proteins. The latter provide resistance to nematode and YVMV infection [22-25]. However, in the test plants were treated with the NE after inoculation with live nematode did not show that much increase in the number of proteins in the root. This shows that nematode infection somehow serves as a repressor for the expression of the defense gene in the test plants [22-25]. This present experiment indicates that nematode extract and acaciasides by acquiring systemic resistance serve as a stimulus for the induced defense responses of the host plants by expression of some new proteins against parasitic nematodes and YVMV infection.

Future Research

And the use of the biomedicines; NE with acaciasides would go a long way in tackling various pests of crops in a safe way by inducing their defense responses of host plants against pathogens and may be controlled by 21st-century pandemic diseases by increasing natural immunity. And in the near future [25], synthetic production of biomedicine-NE will be cost-effective, easily maintainable supply-chains [36], accurate information, and plans for ensuring sustainable developments, continuous supply of essential medicines by improving the biomedicines for the benefit of the global public health [25, 36] with the development of earth-environmental-health-and-research, and in future it may be proved that the "Earth sciences, an "out of system" science: epistemology, models and skills" [37].

Conclusion

Nematode extract and acaciasides use as preventive potential environment-friendly biomedicines against plant diseases by improving agriculture, crop production, toxic-free earth-environmental-health-and-research, science-technology, and communication. And the use of the biomedicines; NE with acaciasides would go a long way in tackling various pests of crops in a safe way by inducing their defense responses of host plants against pathogens and may be controlled by 21st-century pandemic diseases by increasing natural immunity. And in the near future, synthetic production of biomedicine-NE will be cost-effective, easily maintainable supply-chains, accurate information, and plans for ensuring sustainable developments, continuous supply of essential medicines by improving the biomedicines for the benefit of the global public health with the development of earth-environmental-health-and-research.

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