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Efficacy of *Andrographis paniculata* (Burm.f.) Nees against root-knot nematode in pepper, *Piper nigrum* L.

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Abstract
A survey conducted in Thiruvananthapuram, Kollam and Idukki districts in Kerala during 2017-18, revealed that *Meloidogyne incognita* was the most damaging nematode in pepper. As a better alternative to chemicals, preliminary screening of aqueous extracts of weed plants was conducted for their nematicidal properties and selected plant materials were subjected to solvent extraction. Methanolic extract of *Andrographis paniculata, Glyricidia maculata* and *Chromolaena odorata* were found effective against *M. incognita.* Results of pot culture experiments using different plant preparations revealed that *A. paniculata* dried powder @ 50g/kg soil was equally effective to *A. paniculata* dried powder @25g/kg soil in suppressing nematode population in soil and root (88 to 92 per cent reduction over untreated). Micro plot studies confirmed that above treatments are significantly superior to *Purpureocillium lilacinum* and neem cake treatments. Soil amendment with *A. paniculata* dried powder can be recommended as the best substitute for chemical nematicides in managing root knot nematode infestation in pepper.

Keywords: Pepper, *Meloidogyne incognita, Andrographis paniculata, Glyricidia maculata, Chromolaena odorata*, micro plot studies

Introduction
Black pepper (*Piper nigrum* L.), the king of spices is the most traded spice in the world and is being cultivated in humid tropics which require high rainfall and humidity. India is one of the leading producers of the world and Kerala contributes about 35% of total production of the country. Phyto parasitic nematodes are one among the most notorious crop pests infesting black pepper causing an economic loss of 30 to 65 per cent [1]. Although a large number of plant parasitic nematodes have been reported in black pepper, root-knot nematodes (*Meloidogyne sp*) and burrowing nematode (*Radopholus similis*) cause economic damage. The root-knot nematode, *Meloidogyne sp*. was the first nematode reported in black pepper. In 1906, Butler reported root-knot nematode from black pepper in Wayanad, Kerala. *M. javanica, M. incognita* and *M. piperi* have been reported to infect black pepper [2]. Most typical symptom of nematode infection in pepper is yellowing of leaves, spike shedding, cessation of growth and die back. The tender thin white feeding roots infected with *R. similis* show typical orange to purple coloured lesions. The root system exhibit extensive rotting and the main roots are devoid of fine feeder roots that rot quickly. Root systems become heavily galled in the case of root-knot nematode infection and adult females with egg masses are enclosed deep within roots.

Besides direct damage, these nematodes serve as predisposing agents in development of disease complexes with pathogens. Association of *R. similis* with yellow disease of pepper was reported from India [3]. The slow wilt disease of black pepper was reported from Wayanad area in Kerala in 1902. The diseased vines die within three to five years of initiation of yellowing and hence the name slow decline or slow wilt disease. Shedding of spikes is the major symptom in bearing vines. Nematode affected areas become yellow patches and later turn as barren standards or standards supporting dead vines without any leaves. *R. similis* and *Phytophthora capsici* in association resulted root rotting leading to slow decline disease. Synergistic interaction was observed between *M. incognita* and *Fusarium* sp. too [4]. High cost of synthetic chemical pesticides and the risk of nematicidal residues necessitate use of natural plant products for nematode management in spices. Moreover, the chemicals having nematicidal property *viz*. carbofuran and phorate widely used for management of nematodes in

pepper, banana and rice were recently banned necessitating alternative safer nematode management strategies, which have thrown light into the concept of botanical nematicides.

Kerala being a hotspot of biodiversity, there is immense scope for utilizing the floral diversity for extracting biomolecules having nematicidal action. A number of plants including weeds with potential pesticidal properties have not been exploited in an effective way in Kerala. Unlike synthetic pesticides, which are mostly based on single active ingredient, biopesticides have several bio-active components which work together and thus prevent the development of resistant strains and undergo fast dissipation and non bioaccumulating [5]. Nature's chemical factories, the plants offer an excellent source of biologically active natural products which can limit nematode population. In this context, the present investigation aimed to study the distribution of plant parasitic nematodes in pepper growing tracts of Kerala, screening of different plants for their nematicidal properties and evolving an eco-friendly management strategy against the major nematode pest in black pepper.

2. Materials and Methods

2.1. Distribution of plant parasitic nematodes in the rhizosphere of Pepper

A survey was conducted in major pepper growing areas in Thiruvananthapuram, Kollam and Idukki districts of Kerala during 2017-18 to document the nematode fauna associated with pepper. One hundred soil and root samples were collected from the rhizosphere of pepper plants grown in different locations across above mentioned districts. The samples were bulked and 200cc composite soil and 5g root samples from each location were processed by Cobb's sieving and decanting technique followed by modified Baermann's funnel technique [6]. The population of each species of nematode was counted under a stereo zoom microscope. Permanent mounts were prepared for identification of nematodes up to species level. Range, frequency of occurrence and average were estimated from the data obtained.

2.2. *In vitro* screening of plant extracts for nematicidal property

Preliminary screening of aqueous extracts of *Glyricidia maculata*, Panicum maximum, *Tithonea diversifolia*, *Leucas aspera*, *Spilanthus paniculata*, *Wedelia trilobata*, *Samadera indica*, *Carica papaya*, *Chromolaena odorata* and *Mikania micrantha* leaves; *Simarouba glauca* and *Sweitenia mahagoni* seeds; *Andrographis paniculata* leaf and whole plant and Quisqualis indica flower was done for their nematicidal properties against *M. incognita*. Fresh chopped plant materials (100g) were macerated in an electric grinder and immersed in 100 ml of distilled water taken in a reagent bottle. The bottle was shaken well and kept overnight at room temperature. The mixture was then filtered through cheese cloth and Whatman No.1 filter paper in succession. The volume was made up to 100 ml to prepare the stock extract and stored in refrigerator at 4ºC till usage. Ten per cent concentration of aqueous extracts were prepared and tested against one hundred second stage juveniles of *M. incognita* and per cent mortality was recorded 24 hours after treatment.

2.3. *In vitro* screening of solvent extracts of plants for nematicidal property

The selected plant materials were dried and extracted with solvents such as hexane, ethyl acetate and methanol at different concentrations (0.1, 0.5 and 1%). The extracts were fractioned by silica gel column chromatography with petroleum ether: ethyl acetate: methanol of increasing polarity (Table 1). Hundred freshly emerged second stage juveniles of *M. incognita* were suspended in 5ml of each concentration of solvent extracts in sterile vials. All vials were kept in BOD incubator at a temperature of 300C. Per cent mortality was estimated by counting the number of juveniles dead at 24 hours interval after treatment for three days.

Table 1: Mobile Phases of column chromatography

Solvent system	Ratio
Petroleum ether	100
Petroleum ether: Ethyl acetate	80:20
Petroleum ether: Ethyl acetate	60:40
Petroleum ether: Ethyl acetate	40:60
Petroleum ether: Ethyl acetate	20:80
Ethyl acetate	100
Ethyl acetate: Methanol	80:20
Ethyl acetate: Methanol	60:40
Ethyl acetate: Methanol	40:60
Ethyl acetate: Methanol	20:80
Methanol	100

2.4. Pot culture experiment

A pot culture study was conducted to standardize the appropriate preparation and method of application of the selected plants from *in vitro* studies. Eight month old rooted cuttings of pepper (variety-Panniyur 8) maintained as per Package of Practices Recommendations of Kerala Agricultural University were selected for pot culture experiment. Newly hatched second stage juveniles of *M. incognita* were inoculated to the pepper rhizosphere as per the method suggested by Venkitesan and Sethi [7]. The experiment was laid out in CRD with ten treatments and three replications. Effect of different treatments viz. soil application of extract absorbed charcoal in granular form@25 and 50g/kg soil, soil application of dried powder @25 and 50g/kg soil and soil drenching of crude extracts @25 and 50 ml/plant was evaluated. Control plants also maintained. Observations on nematode population in soil (200 cc) and root (5g) and number of galls in 5g root were recorded after uprooting the plants 45 days after planting.

2.5. Micro plot studies

A micro plot study was conducted to evaluate the efficacy of botanical selected from pot culture study in comparison with recommended chemical (carbosulfan @ 1 Kg a.i. /ha), bio

agent (*Purpureocillium lilacinum* @ 20g/m2) and organic amendment (neem cake @ 200g/m2). The trial was laid out in CRD with four replications in micro plots of 6x6m size in College of Agriculture, Vellayani with initial population of 350-420 *M. incognita* juveniles/ 200 cc soil. Eight month old rooted cuttings of pepper (variety Panniyur-7) with uniform growth were planted in nematode infested sick plots at a spacing of 3x3m. A control plot was also maintained. Observations were taken on nematode population in soil and roots and number of galls in pepper roots.

2.6. Statistical analysis
The data generated from the experiments were subjected to analysis of variance (ANOVA) technique [8]. The variables which did not satisfy the basic assumptions of ANOVA were subjected to angular and square root transformations before analysis.

3. Results and Discussion
The results of survey revealed the presence of root-knot nematode, *M. incognita*, Reni form nematode, *Rotylenchulus reniformis*, burrowing nematode, *R. similes*, spiral nematode, *Helicotylenchus* sp. and stunt nematode, *Tylenchorhynchus* sp. in the rhizosphere of pepper plants grown in Thiruvananthapuram, Kollam and Idukki districts (Table 2). In Amboori area of Thiruvananthapuram district highest population of *M. incognita* was observed which ranged from 39-728 with an average of 329 and frequency occurrence of 100. Next predominant nematode in Amboori area was *R. reniformis* with population range of 54-710, average of 498.75 and frequency of occurrence of 100. The population of other plant parasitic nematodes viz., *Tylenchorhynchus* sp. and *H. dihystera* ranged from 0-150 and 0-359 with an average of 25 and 144.14 respectively. In Palode area of Thiruvana tha puram district population of *M. incognita* ranged from 0-150 with an average of 25 and frequency of occurrence of 30.8. Population of *H. dihystera* ranged from 0-490 with an average of 98.33 and frequency of occurrence of 20. In Peringamala area of Thiruvananthapuram district the population of *M. incognita* ranged from 0-465 nematodes/200 cc soil with an average of 129 and frequency of occurrence of 25. In Vellarada area of Thiruvananthapuram district, presence of *M. incognita*, *R. similis* and *Tylenchorhynchus* sp. was observed with population range of 0-118, 0-8 and 0-271 nematodes per 200cc soil respectively.

M. incognita, *H. dihystera*, *R. reniformis* and *Tylenchorhynchus* sp. were the plant parasitic nematodes present in the rhizosphere of pepper in Kollam district. Highest population of *M. incognita* (51-725/200cc soil) was observed in Kulathuppuzha area. Other plant parasites observed were *R. similis* and *Tylenchorhynchus* sp. with population range of 5-10 and 0-180 nematodes/200cc soil respectively. Population of *M. incognita* ranged from 39-538, 50-300 and 0-234 nematodes per 200cc soil in Pinatin mukal, Valakam and Ummannoor areas of Kollam district respectively. Samples collected from Pinatinmukal and Valakam areas showed presence of *R. reniformis* with population range of 11-153 and 0-79 nematodes per 200cc soil respectively. Other than *M. incognita*, occurrence of H. dihystera and *Tylenchorhynchus* sp. was observed in Ummannoor area with population range of 16-621 and 0-79 nematodes/200cc soil respectively.

In Pampa dumper area of Idukki district, the population of *M. incognita* ranged from 0-714 with an average of 158.4 and frequency of occurrence of 75. Other than *M. incognita*, population of *H. pseudorobustus*, *R. similis* and *R. reniformis* ranged from 0-218, 4-21 and 0-310 respectively. In Nedumkandam area of Idukki district presence *M. incognita* (0-250/200cc soil) and *Tylenchorhynchus* sp. (0-150 nematodes/200cc soil) was observed. Other than *M. incognita*, presence of *H. pseudorobustus*, *R. similis*, *R. reniformis* and *Tylenchorhynchus* sp. was observed in samples collected from pepper grown in Panikamkudi area in Idukki district with population range of 0-146, 0-16, 0-215 and 11-153 respectively. Samples collected from Rajakkad area of Idukki district showed presence of *H. pseudorobustus* (10-225 nematodes/200cc soil), *R. similis* (0-29 nematodes/200cc soil) and R. reniformis (50-280 nematodes/200 cc soil) other than *M. incognita* with population range of 10-225, 0-29 and 50-280.

The findings of the survey revealed that *M. incognita* is the most abundant nematode in pepper growing areas of Kerala with frequency of occurrence ranging from 30 to 100. This was in tune with the study of Ramana *et al*., who conducted a survey in pepper growing tracts of Kerala and reported 14 genera of plant parasitic nematodes associated with black pepper, of which *M. incognita* and *R. similis* were recorded as the major prevalent nematodes [9]. Considering the distribution and mode of parasitism, *M. incognita* was the most challenging nematode in pepper. To evolve an eco-friendly management strategy, *in vitro* screening studies were conducted to evaluate the nematicidal property of aqueous extracts of weed plants which are available plenty in Kerala.

Table 2: Nematode population in the rhizosphere of Pepper in Thiruvananthapuram, Kollam and Idukki District.

District	Location	Nematode population (200g soil) Range: Average Frequency of Occurrence				
		Meloidogyne incognita	***Helicotylenchus sp.***	***Radopholus similis***	***Rotylenchulus reniformis***	***Tylenchorhynchus sp.***
Thiruvananthapuram	Amboori	39-728 :329 100	0-359:144.1 83.33		54-710:498.75 100	0-150:25 70
	Palode	0-150 : 25 30.8	0-490:98.33 20			
	Peringamala	0-465 :129 50		0-3: 0.75 25	0-150: 61.00 40	0-170 :66 53.9
	Vellarada	0-118 : 29 38.5		0-8 :2.88 62.5		0-271:150 63
Kollam	Valakam	50-300 :110 67	57-714:334.8 100		0-79: 23.83 40	
	Ummannoor	0-234:84.13 75.0	16-621:262.33 100			0-79:23.83 40
	Kulathuppuzha	51-725:405.3 100		5-10:3.00 40		0-180 :95.5 83.33
	Pinatinmukal	39-538:285.9 100			11-153:89.33 100	54-728 :498.7 100
Idukki	Pampadumpara	0-714:158.4 75	0-218:54.5 25	4-21:6.25 100	0-310:104 53.9	
	Nedumkandam	0-250:88.5 60				0-150 : 61.00 40
	Panikamkudi	0-100:69.67 40	0-146 : 82 75	0-16 : 6.5 50	0-215:83 80	11-153 :89.33 100
	Rajakkad	0-238:65.88 62.5	10-225:107 50	0-29 :7.25 25	50-280:66.25 100	

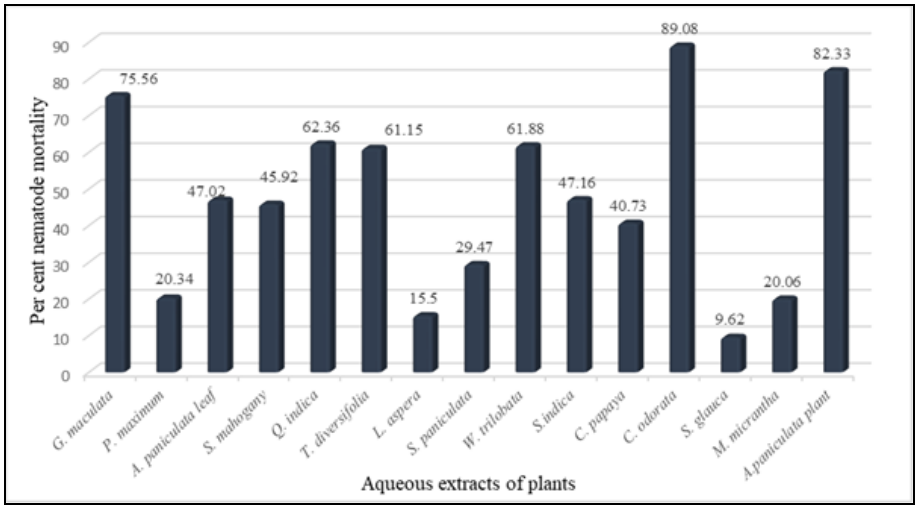

Fig 1: *In vitro* screening of aqueous plant extracts against second stage juveniles of *M. incognita* at 24 hours after treatment

Among the different aqueous plant extracts tested, all extracts at 10 per cent concentration showed significant nematicidal properties against second stage juveniles of *M. incognita* (Fig. 1). Maximum nematode mortality (89.08%) was recorded by C. odorata leaf extract followed by *A. paniculata* plant extract (82.33%) and *G. maculata* leaf extract (75.56%) at 24 hours after treatment. Next best treatments in the order of effectiveness were *Q. indica* flower extracts, *W. trilobata* and *T. diversifolia* leaf extracts giving percentage mortality of 62.36, 61.88 and 61.15 respectively. Minimum nematicidal action (9.62%) was observed in *S. glauca* seed extracts. The aqueous plant extracts which showed more than 75 per cent nematicidal activity were subjected to solvent extraction process. Potential of aqueous plant extracts in suppressing nematode population was earlier reported by many investigators unravelling its future scope in nematode pest management. The bio efficacy of aqueous extracts of *C. odorata* in nematode management was earlier proved by Adegbite and Adesiyan, who reported that aqueous extracts of *C. odorata* roots at 100% concentration showed cent per cent inhibition of egg hatch and larval mortality of *M. incognita* [10]. Ferris and Zheng reported that *M. javanica* and Pratylenchus vulnus when exposed to aqueous extracts of A. paniculata, became inactive rapidly with non-recovery response patterns [11].

Among the different solvent extracts of selected plant materials, maximum nematicidal action was shown by methanol extracts of *A. paniculata* plant at 1 per cent concentration (97.67%) against second stage juveniles of *M. incognita* at 72 hours after treatment (Table 3). One per cent methanol extract of *G. maculata* leaves and *C. odorata* leaves exhibited nematicidal activity in the range of 95 to 97% and 86.67 to 92% respectively. Minimum nematicidal action was shown by hexane extracts of *A. paniculata*, *G. maculata* and *C. odorata*. The findings of the above *in vitro* experiments clearly showed that methanol extracts of *A. paniculata*, *G. maculata* and *C. odorata* are superior in nematicidal action compared to

Table 3: Nematicidal effect of different solvent extracts against *M. incognita*

Treatments		*A. paniculata* plant			*G. maculata* leaf			*C. odorata* leaf		
Solvent extract	**Concentration**	**24 HAT**	**48 HAT**	**72 HAT**	**24 HAT**	**48 HAT**	**72 HAT**	**24 HAT**	**48 HAT**	**72 HAT**
Hexane extract	0.1%	65.00^{e} (53.75)	69.00^{e} (56.84)	73.33^{e} (58.96)	64.33^{f} (53.35)	66.67^{g} (54.76)	73.33^{f} (58.98)	45.00^{f} (42.13)	51.33g (45.76)	55.67^{f} (48.26)
Methanol extract		78.00^{bc} (62.05)	82ef.33^{bc} (65.18)	85.67^{bc} (67.89)	77.67^{cd} (61.82)	80.67^{de} (63.92)	83.33^{de} (65.91)	68.33^{c} (55.85)	71.67^{cd} (57.99)	75.67^{cd} (60.69)
Ethyl acetate extract		68.30^{de} (55.76)	73.33^{de} (58.93)	78.33^{de} (62.29)	73.33^{de} (58.96)	76.67^{ef} (61.15)	82.00^{e} (64.92)	53.33^{e} (46.92)	57.67^{efg} (49.41)	62.00^{ef} (51.95)
Hexane extract	0.5%	71.00^{de} (57.45)	75.67^{cde} (60.56)	78.00^{de} (62.17)	72.33^{de} (58.28)	77.00^{ef} (61.37)	82.67^{de} (65.45)	52.33^{ef} (46.34)	57.33^{fg} (49.23)	62.00^{ef} (51.97)
Methanol extract		82.33^{b} (65.18)	85.67^{b} (67.89)	88.33^{b} (70.12)	86.67^{b} (68.60)	90.00^{b} (71.62)	92.00^{b} (73.65)	77.67^{b} (61.82)	82.00^{b} (64.92)	84.67^{b} (67.02)
Ethyl acetate extract		74.30^{cd} (59.60)	78.00^{cde} (62.13)	80.33^{cde} (63.77)	82.00^{bc} (64.92)	84.67^{cd} (67.02)	86.67^{cd} (68.67)	59.00^{de} (50.19)	61.33^{ef} (51.56)	66.67^{e} (54.75)
Hexane extract	1%	73.00^{cd} (58.71)	76.67^{cde} (61.17)	79.00^{cde} (62.78)	83.67^{b} (66.18)	88.00^{bc} (69.78)	90.33^{bc} (71.92)	62.00^{cd} (51.97)	66.00^{de} (54.38)	70.00^{de} (56.84)
Methanol extract		94.33^{a} (76.84)	96.00^{a} (79.10)	97.67^{a} (81.53)	95.00^{a} (77.82)	96.00^{a} (79.10)	97.00^{a} (80.27)	86.67^{a} (68.60)	90.00^{a} (71.62)	92.00^{a} (73.65)
Ethyl acetate extract		78.30^{bc} (62.29)	81.33^{bcd} (64.46)	83.00^{bcd} (65.78)	69.00^{ef} (56.17)	72.00^{fg} (58.06)	75.00^{f} (60.03)	72.67^{b} (58.59)	74.67^{bc} (59.91)	79.67^{bc} (63.30)
CD (0.05)		(4.37)	(5.56)	(5.29)	(4.230)	(4.116)	(3.512)	(4.774)	(5.06)	(5.28)

Figures given in the parenthesis are values after angular transformation

Other solvent extracts. Nematicidal effect of methanol chloroform extracts of *A. paniculata* against *M. incognita* was reported by Devi [12]. Methanolic extracts of A. paniculata found to be highly effective against *M. javanica* at 1:5 concentration [13]. The biocidal effect of methanol extracts of *C. odorata* against insect pests have been reported, while its

effect on plant parasitic nematodes is less investigated [14]. Perez *et al*. (2014) reported the anthelminthic properties of methanolic extract of *G. sepium* [15].

The present study succeeded to confirm the nematicidal properties of both aqueous and methanol extracts of *A. paniculata*, *G. maculata* and *C. odorata*. Aqueous extracts of these plants exhibited more than 75 per cent mortality against J2 stage of *M. incognita* at 24 hours after treatment, while the methanolic extracts of *A. paniculata* and *G. maculata* showed more than 90 per cent and *C. odorata* exhibited more than 85 per cent nematicidal activity respectively. Hence these plants were selected for standardizing the appropriate preparation and method of application viz., soil application of methanol extract adsorbed charcoal in granular form @ 25 and 50g/Kg soil, soil application of dried powder@ 25 and 50g/Kg soil and soil drenching of crude extracts @ 25 and 50 ml/plant under pot culture condition.

Table 4. Effect of different treatments on nematode population characteristics of *M. incognita* in Pepper under pot culture condition

Treatments			Population of nematodes		Number of galls
Preparations	**Plants**	**Dose**	**Soil (200cc)**	**Root (5g)**	**(5g root)**
Methanol extract absorbed charcoal granules	*A. paniculata*	25g/Kg soil	136.67 ^{h} (11.68)	41.00^{fg}(6.39)	20.00^{fgh}(4.50)
		50g/Kg soil	121.67 ^{efgh} (11.02)	26.67^{cde}(5.14)	17.33^{defgh}(4.13)
	G. maculata	25g/Kg soil	101.67 ^{cdef} (10.07)	22.67^{cd}(4.74)	17.67^{efgh}(4.19)
		50g/Kg soil	86.33^{bc} (9.29)	15.67^{b}(3.93)	14.67^{def}(3.77)
	C. odorata	25g/Kg soil	104.67 ^{cdef} (10.23)	28.00^{de}(5.28)	20.00^{fgh}(4.46)
		50g/Kg soil	84.00 ^{bc} (9.16)	21.00^{bcd}(4.57)	17.67^{efgh}(4.19)
Dried powder form	*A. paniculata*	25g/Kg soil	50.67 ^{a} (7.09)	6.67^{a}(2.53)	1.33^{a}(1.13)
		50g/Kg soil	42.67 ^{a} (6.49)	5.00^{a}(2.16)	1.00^{a}(1.00)
	G. maculata	25g/Kg soil	94.00^{bcd}(9.68)	37.67^{fg}(6.11)	12.33^{cde} (3.50)
		50g/Kg soil	79.67^{b}(8.92)	32.67^{ef}(5.69)	11.67^{bcd}(3.38)
	C. odorata	25g/Kg soil	99.33 ^{bcde} (9.96)	34.00^{ef}(5.82)	9.00^{bc}(2.95)
		50g/Kg soil	94.33 ^{bcd} (9.71)	20.00^{bc}(4.46)	7.67^{b}(2.71)
Soil drenching of crude extracts	*A. paniculata*	25ml/plant	133.33 ^{gh} (11.53)	25.67^{cde}(5.05)	22.00^{gh}(4.68)
		50ml/plant	116.00^{defgh} (10.75)	21.00^{bcd}(4.57)	20.00^{fgh}(4.46)
	G. maculata	25ml/plant	123.33^{fgh}(11.08)	40.00^{fg}(6.31)	23.33^{h}(4.80)
		50ml/plant	109.33^{defg}(10.45)	32.00^{ef}(5.64)	19.33^{fgh}(4.38)
	C. odorata	25ml/plant	120.00^{efgh}(10.91)	45.00^{g}(6.70)	15.67^{defg}(3.93)
		50ml/plant	115.33^{defgh}(10.74)	27.00^{cde}(5.18)	14.33^{def}(3.75)
Untreated			416.67^{i}(20.40)	62.67^{h}(7.89)	33.33^{i}(5.75)
CD(0.05)			(1.081)	(0.795)	(0.763)

Figures given in the parenthesis are values after square root transformation

The data from pot culture studies on the effect of application of different treatments of *A. paniculata*, G. maculata and *C. odorata* on the population buildup of nematodes in the rhizosphere of pepper showed significant variation (Table 4). Plants treated with soil application of A. paniculata dried powder @ 50g/Kg soil showed lowest mean nematode population in soil (42.67) and root (5.00) and minimum number of galls (1.00) in pepper roots. Effect of this treatment was found on par with soil application *A. paniculata* dried powder @25g/kg soil. These two treatments were found significantly superior to all other treatments in reducing number of root galls and nematode population in soil and root. The next best treatments in reducing soil and root nematode population were soil application of *G. maculata* dried powder @ 50g/Kg soil and application of methanol extract absorbed charcoal granules of *G. maculata* @ 50g/Kg soil respectively. Soil application of *C. odorata* dried powder @ 50g/Kg soil was found effective in reducing number of pepper root galls (7.67) next to *A. paniculata* dried powder @25g/kg soil. The potential of these plants in managing plant parasitic nematodes was earlier reported by many investigators. Application of *G. maculata* as green leaf manure on tomato under field conditions exhibited minimum number of root galls, gall index and reproductive factor of *M. incognita* and promoted plant growth [16]. The chopped leaves of *G. maculata* @ 10g/Kg soil as green manure reduced the population of *R. similis* and promoted the growth of black pepper under pot culture conditions [17]. Application of *C. odorata* leaves as organic mulch @ 10 tons/ha significantly lowered the number of root galls (28.16 galls/plant) in *Celosia argentea* compared to control treatment (110.83 galls/plant) [18].

The results of pot culture experiment revealed that soil application of *A. paniculata* dried powder @ 25 and 50 g/Kg was the superior treatments with significant reduction in soil and root nematode population and in number of pepper root galls. Possibility of dried powder application of plants for nematode management was earlier researched. Leaf powder of Myrtus communis added to sand at 0.1% (w/w) reduced the number of juveniles of *M. javanica* recovered from the sand by more than 50 per cent, while the leaf powder when incorporated in soil @ 0.1-0.4% (w/w) reduced galling index and number of nematode eggs in tomato roots during pot culture studies [19]. The finding of the present study on the efficacy of soil application of *A. paniculata* dried powder for managing root-knot nematode in pepper rhizosphere is the first of its kind being reported so far.

Based on the results of pot culture experiment, soil application of *A. paniculata* dried powder @ 25 and 50 g/Kg soil was identified as the best treatment and tested in micro plot condition to confirm the nematicidal property (Table 5). Dried powder of *A. paniculata* @ 50g/plant showed significantly higher reduction of nematode population in soil (80.16%), root (62.23%) and number of galls (68.70%). This treatment was on par with dried powder of *A. paniculata* @ 25g/plant showing 78.02, 59.84 and 57.39 per cent reduction in nematode population in soil and root and gall formation respectively. The effect of dried powder of *A. paniculata* was found superior to the effect of the bio agent, *P. lilacinum* @ 20g/m2 and neem cake @200g/m2.

Table 5: Effect of different treatments on nematode population in Pepper rhizosphere

Treatments	Nematode population in soil (200g)	Per cent reduction over untreated	Nematode population in root (5g)	Per cent reduction over untreated	No. of Galls	Per cent reduction over untreated
Dried powder of *A. paniculata* @25g/ plant	87.25[b] (9.34)	78.02	37.75[a] (5.48)	59.84	12.25[bc] (3.49)	57.39
Dried powder of *A. paniculata* @50g/ plant	78.75[b] (8.87)	80.16	35.50[a] (5.95)	62.23	9.00[b] (2.85)	68.70
Carbosulfan @1kg a.i./ha	67.50[a] (8.18)	83.00	31.25[a] (5.59)	66.76	4.75[a] (2.12)	83.48
Purpureocillium lilacinum@20g/m^2	83.50[b] (9.13)	78.97	45.25[b] (6.72)	51.86	12.50[bc] (3.48)	56.52
Neem cake @200g/m^2	87.00[b] (9.32)	78.09	45.50[b] (6.74)	48.50	16.75[c] (4.05)	41.74
T6-Untreated	397.00[c] (19.92)		94.00[c] (9.69)		28.75[d] (5.36)	
CD value	(0.651)		0.618		(0.695)	

Figures given in the parenthesis are values after square root transformation

This study recommends soil application of *A. paniculata* dried powder as a better alternative to chemical nematicides in managing root knot nematode in pepper. The bio efficacy of this plant is well investigated by many researchers and reported its various mode of biological activities viz., immunostimulatory, antiviral, anti-cancer, anti-inflammatory and antibacterial activities [20]. The active fractions from the acetone extract of A. paniculata leaves and stem exhibited antifeedant and Oviposition deterrent effect on diamond back moth, *Plutella xylostella* [21]. Further studies confirmed presence of a bioactive component, 14-deoxyandrographolide responsible for its bio efficacy against P. xylostella [22].

There is immense scope for exploiting this annual herbaceous weed plant for nematode management, yet only few attempts were made in this area. The nematicidal action of A. paniculata against *M. incognita* on tomato was researched by Goswami and Vijayalakshmi who reported the potential of A. paniculata in reducing nematode population and number of galls in tomato roots under pot culture experiments [23]. Jumaah reported the nematicidal action of *A. paniculata* against *M. incognita* in *Solanum melongena* [24]. The effect of chloroform methanol extracts of *A. paniculata* on egg hatch inhibition and larval mortality of *M. incognita* was reported by Devi and Simon [25]. Singh and co-workers researched on the effect of A. affinis against *M. incognita* and reported that crude extracts of A. affinis exhibited 99.46 per cent mortality against J2 stages of *M. incognita* in tomato at 72 hours after treatment and the phytochemical screening of extract confirmed the presence of glycosides, triterpenoids, flavonoids, alkaloids and steroids [26].

4. Conclusion

The findings of the present investigation has thrown light into the immense potential of locally available weed plants. Both the aqueous and solvent extracts of many plants exhibited promising nematicidal activity. The pot culture studies showed the bio efficacy of different preparations of A. paniculata, *C. odorata* and *G. maculata* against second stage juveniles of *M. incognita*. Among these the most effective treatments observed were dried powder application of *A. paniculata* @ 25 and 50 g/ plant with more than 80 per cent reduction of soil nematode population. Since both the treatments are statistically on par, considering economy, application of dried powder of A. paniculata @ 25 g/plant can be considered as the best treatment and it was found superior to *P. lilacinum* and neem cake treatments. Hence soil amendment with dried powder of *A. paniculata* @ 25 g/plant can be recommended as the best substitute for chemical nematicides in managing root knot nematode infestation in pepper.

5. References

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