D-5269
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RESEARCH ARTICLE Agricultural Science Digest, Volume Issue : ()

Control of Root-knot Nematode, *Meloidogyne incognita* and Reniform Nematode, *Rotylenchulus reniformis* on Chickpea

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10.18805/ag.D-5269

ABSTRACT

Background: The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood and reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveiro) have been found consistently associated with plant damage and reduced yield of chickpea (*Cicer arietinum* L.), important pulse (grain legume) crop of India.

Methods: Seeds of chickpea were soaked in S, S/2, S/4 extracts of rice polish and 0.1, 0.3, 0.5% solution of pyridoxine for 4 h. Four treated seeds were sown in each pot. Three week old plants were inoculated with 5000 *M. incognita* or *R. reniformis* larvae.

Result: The root-knot nematode *M. incognita* and reniform nematode, *R. reniformis* caused significant reduction in different growth parameters (length and weight of plant, number of pods), chlorophyll content of leaf and water absorption of roots. Similar effects were also observed in plants raised from seeds soaked in different concentrations of water soluble fractions (WSF) of rice polish and pyridoxine solutions, however, the reductions were of comparatively lesser extent. Higher concentrations of the solutions were more effective as compared to lower ones and pyridoxine was more beneficial than WSF for improving plant growth and reducing disease incidence.

Key words: *Cicer arietinum, Meloidogyne incognita,* Pyridoxine, Reniform nematode, Rice polish, Root-knot nematode, *Rotylenchulus reniformis.*

INTRODUCTION

A survey conducted in the Aligarh district revealed the presence of the root-knot, *Meloidogyne incognita* (Kofoid and White) Chitw. and reniform nematode, *Rotylenchulus reniformis*, (Linford and Oliveira) on chickpea. These nematodes have been reported to be potential constraints to the productivity of this crop (Anver and Alam 1994 and 1995). Thus a breakthrough in the yield of test crops can be achieved by adopting suitable measures of disease management through advanced scientific technology.

Non-chemical control strategies involve biological and cultural methods such as the use of nematode pathogenic organisms, organic amendments and use of nematode resistant cultivars (Anver and Alam, 1999 and 2000; Anver, 2006; Tiyagi and Ajaz, 2004; Ashraf and Khan, 2010; Mahalik and Sahoo, 2019). Water soluble fractions (WSF) of oil-seed cakes of mahua, castor, mustard, neem and peanut and their mixtures with unsterilized soil were reported to be highly deleterious to *M. incognita, R. reniformis* and *Tylenchorhynchus brassicae* (Alam *et al*. 1982). Also, an increase in the period of decomposition of oil-seed cakes and cake-soil mixture enhanced the toxicity of the WSF. Similarly, the WSF of oil-seed cakes significantly inhibited the larval hatching in *M. incognita* (Khan *et al*. 1974). In the present study, the effect of seed-soaking treatment with rice polish and pyridoxine was tested on the root-knot development of *M. incognita* and final population of *R. reniformis* and growth parameters of chickpea.

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How to cite this article: Anver, S. (2022). Control of Root-knot Nematode, *Meloidogyne incognita* and Reniform Nematode, *Rotylenchulus reniformis* on Chickpea. Agricultural Science Digest. DOI: 10.18805/ag.D-5269.

Submitted: 19-10-2020 **Accepted:** 12-09-2022 **Online:** 14-11-2022

MATERIALS AND METHODS

For obtaining water soluble fraction (WSF), 20 g of rice polish was soaked in 200 ml of distilled water in a flask. After vigorous shaking, an extract was obtained by filtering through Whatman filter paper No. 1. The solution was taken as the standard solution 'S'. By adding an appropriate amount of distilled water, S/2 and S/4 concentrations were made. Since WSF of rice polish is rich in pyridoxine, the above experiment was repeated using 0.1, 0.3 and 0.5% solution of pyridoxine hydrochloride in distilled water.

Seeds of chickpea were soaked in S, S/2, S/4 extracts of rice polish and 0.1, 0.3, 0.5% solution of pyridoxine for 4 h. Seeds soaked in distilled water served as control. Fifteen cm clay pots were filled with 1 kg soil (clay, sand and compost mixture in the ratio of 7:2:1) and then autoclaved. Four treated seeds were sown in each prepared pot. After germination, thinning was done and only one plant per pot was allowed to grow. Three-week-old plants were inoculated with 5000 J_2 or immature females of *M. incognita* or *R. reniformis* respectively. Uninoculated plants served as a control.

The experiments were terminated three months after inocu1ation. Plants were uprooted and the roots were thoroughly washed with running tap water. The length (in cm) and fresh/dry weights (in g) of plants were taken separately. For determining the dry weight, plants were first dried in an oven at 60°C and then weighed. The water absorption capability of roots was determined by the method described by Alam *et al*. (1974). The chlorophyll content of the leaves was determined by the method described by Hiscox and Israelstam (1979). The root-knot infection was assessed accordingly to the rating scale (0-5) of Taylor and Sasser (Sasser *et al*. 1984).

In the case of soil population of the reniform nematode, soil from each treatment was processed after the termination of the experiment using Cobb's sieving and decanting and the modified Baerman funnel techniques (Southey, 1986). Statistical analysis of the data for critical difference (C.D.) at P = 0.05 and P = 0.01 levels was done as per procedure described by Pansey and Sukhatme (1978).

RESULTS AND DISCUSSION

The present experiment was conducted to evaluate the effect of seed-soaking in S, S/2, S/4 concentrations of water soluble fractions of rice polish and 0.5%, 0.3%, 0.1% concentrations of pyridoxine (Vit. B_6) solution on different parameters of chickpea infected with the root-knot nematode, *M. incognita*.

Plants of chickpea inoculated with the test pathogen showed significant reduction in different growth parameters (length and weight of plant, number of pods), chlorophyll content of leaf and water absorption capacity of roots. Similar effects were also observed in plants raised from seeds soaked in different concentrations of WSF of rice polish and pyridoxine solutions. However, the reductions were of comparatively lesser extent. Higher concentrations of the solutions were more effective as compared to lower ones and pyridoxine was more beneficial than WSF for improving plant growth and reducing disease incidence (Table 1).

Another experiment was conducted to evaluate the effect of seed-soaking in different concentrations of WSF of rice polish and pyridoxine solution on different parameters of chickpea infected with *R. reniformis*. Its effect on population build up of *R. reniformis* was also studied.

Chickpea was found to be susceptible to the reniform nematode. Reduction in different growth parameters (length and weight of plant, number of pods), chlorophyll content of leaf and water absorption of chickpea roots caused by the *R. reniformis* were statistically significant. Plants raised from treated seeds showed better growth and were less vulnerable to the disease incidence. Seed-soaking in higher concentrations of the above-mentioned solutions was more effective than in the lower ones. Maximum reduction in disease incidence was observed in plants raised from seeds soaked in 0.5% pyridoxine solution and a minimum reduction was observed in plants raised from S/4-WSF of rice polish treated seeds (Table 2).

It is known that vitamins are organic compounds synthesized by plants and required in trace amounts for normal growth and development of organisms. B-vitamins constitute a heterogenous group of varied organic compounds; they are designated for simplification as B_1, B_2, B_6, *etc.* They act not only as coenzymes and phytochromones (Bonner and Bonner 1948) but also play a regulatory role in various physiological processes. Some B-vitamins promote respiratory activity of many plants (DeCapite 1949). B-vitamins are reported to be involved in chlorophyll synthesis (Kodandaramaiah and Gopala Rao 1984) and protein synthesis either at transcriptional or translational level (Gopala Rao 1973). Regulation of enzyme activity by B-vitamins is well established (Lehninger 1982). Kodandaramaiah and Gopala Rao (1985) found an increase n the stomatal index due to the application of some vitamins 'of the B complex group. In the present experiments, the exogenous application of WSF of rice polish and pyridoxine (vitamin B6) solution improved the growth of un-inoculated plants over untreated un-inoculated plants (Table 1 and 2). The plant response to the exogenous application of different growth regulatory substances including B-vitamins, which may improve the germination of seeds and growth of seedlings, depends on its need for these substances. Bonner and Greene (1938) studied many crop responses towards thiamine (vitamin B_1) application. Crops with low endogenous levels of vitamin B_1 responded positively to the vitamin treatment, while crops having a high content of the vitamin proved to be non-responsive. Improved plant growth due to the application of different B-vitamins has also been obtained by other workers. Sinkovics (1974) applied seven different B-vitamins to two cultivars of capsicum through seed-soaking and secured high seed germination rates coupled with improved seedling growth. Barbieri (1959) observed that the application of vitamins B_1 and B_2 enhanced plant height, leaf number, fresh and dry weight of pea, broad bean, beet and wheat in pot culture. Afridi *et al*. (1985) and Ansari and Khan (1986) observed pyridoxine-mediated enhancement in plant growth in *Vigna radiata*. Ahmad (1975) reported the usefulness of seed-soaking for 4 h in pyridoxine solution for an improvement in growth, seed yield, oil yield and quality of mustard oil. The present study revealed that seed-soaking in different concentrations of WSF of rice polish and pyridoxine solution brought about significant reduction in root-knot development and multiplication of *R. reniformis* with a corresponding increase in plant growth characters and water absorption capacity of roots. Higher concentrations of WSF of rice polish and pyridoxine solutions were more effective in reducing the damage caused by nematodes as compared to lower concentrations. Treatments with pyridoxine solutions were more effective than WSF treatment. It has been known for many crops that seed treatment with very dilute solutions of B-vitamins

Table 1: Effect of seed-soaking in water soluble fraction (WSF) of rice polish and pyridoxine solution on the root-knot development caused by *Meloidogyne incognita* and plant growth of chickpea (*Cicer arietinum*) cv. K-850 (Mean of 5 replicates).

Treatment		Length (cm)		Fresh weight (g)		Dry weight (g)		No. of	Chlorophyll	Root-knot	Water absorbed/
WSF/ Pyridoxine (Conc.)	Mi	Shoot + Root	% reduction	Shoot + Root	% reduction	Shoot + Root	% reduction	pods/ plant*	content (mg/g) (a+b)	index (0-5) scale	plant (g/day)
Untreated	Un	98.5	-	40.0	-	9.5	-	24	2.63	-	38.7
″	In	59.0	40.10	21.4	46.50	5.1	46.31	10	1.19	4.2	22.5
WSF (S)	Un	115.1	-	43.1	-	11.2	-	38	3.15	-	41.8
″	In	85.4	25.80	31.1	27.84	7.8	30.35	21	2.21	2.2	28.0
(S/2)	Un	105.0	-	41.0	-	10.1	-	32	2.90	-	39.7
″	In	69.5	33.80	26.7	36.42	6.1	39.60	20	2.15	3.0	24.6
(S/4)	Un	99.0	-	40.0	-	9.5	-	30	2.70	-	38.0
″	In	60.9	38.48	24.0	40.00	5.5	42.10	15	2.05	3.9	23.1
Pyridoxine	Un	120.6	-	47.8	-	14.4	-	40	3.38	-	43.0
(0.5%)	In	93.6	22.38	36.5	23.64	10.4	27.77	24	2.46	1.5	32.6
(0.3%)	Un	109.8	-	43.6	-	12.0	-	36	3.32	-	40.1
″	In	79.6	27.50	31.0	28.89	8.5	29.16	26	2.21	2.2	26.0
(0.1%)	Un	100.8	-	40.1	-	9.6	-	32	3.15	-	38.9
″	In	70.1	30.45	24.6	38.65	5.7	40.62	18	2.02	3.5	23.7
C.D. (P = 0.05)		3.10		1.49		0.90		3.33	0.41		2.10
(P = 0.01)		4.19		1.49		1.21		4.55	0.57		2.84

Inoculum level of *M. incognita* (Mi) = 5000 J_2/pot.
WSF = Water soluble fraction of rice polish.
Un = Uninoculated; In = Inoculated.
*Rounded off to the nearest whole number.

Table 2: Effect of seed-soaking in water soluble fraction (WSF) of rice polish and pyridoxine solution on the final population of *Rotylenchulus renioformis* and plant growth of chickpea (*Cicer arietinum*) cv. K-850 (Mean of 5 replicates).

Treatment		Length (cm)		Fresh weight (g)		Dry weight (g)		No. of	Chlorophyll	Root-knot	Water absorbed/
WSF /Pyridoxine (Conc.)	Mi	Shoot + Root	% reduction	Shoot + Root	% reduction	Shoot + Root	% reduction	pods/ plant*	content (mg/g) (a+b)	index (0-5) scale	plant (g/day)
Untreated	Un	98.5	-	40.0	-	9.5	-	24	2.63	-	38.7
″	In	65.2	33.80	24.5	37.97	5.8	38.94	12	1.99	23001	24.2
WSF (S)	Un	115.1	-	43.1		11.2		38	3.15	-	41.8
″	In	90.0	21.80	33.0	23.43	8.2	26.78	23	2.25	17500	30.0
(S/2)	Un	105.0		41.0		10.1		32	2.90	-	39.7
″	In	75.5	28.09	29.0	29.26	6.8	32.67	21	2.26	19656	26.0
(S/4)	Un	99.0		40.0		9.5		30	2.70	-	38.0
″	In	6.5	30.80	26.0	35.00	6.0	36.84	17	2.15	22959	25.2
Pyridoxine	Un	120.6		47.8		14.4		40	3.38	-	43.0
(0.5%)	In	95.5	20.81	37.0	22.59	11.0	23.61	26	2.49	12050	34.8
(0.3%)	Un	109.8		43.6		12.0		36	3.32	-	40.1
″	In	81.5	25.70	32.0	26.60	8.6	28.33	28	2.29	15550	28.1
(0.1%)	Un	100.8		40.1		9.6		32	3.15	-	38.9
″	In	72.2	25.70	26.1	34.91	6.0	37.50	20	2.10	21000	25.1
C.D. (P = 0.05)		2.60		1.10		0.90		3.29	0.43		1.70
(P = 0.01)		3.52		1.49		1.21		4.49	0.56		2.29

Inoculum level of *R. reniformis* (Rr) 5000 immature females/pot.
WSF = Water soluble fraction of rice polish.
Un = Uninoculated; In = Inoculated.
*Rounded off to the nearest whole number.

stimulated the growth of roots and thus helped the plants to establish and grow efficiently (Afridi *et al*., 1979). Of the vitamins tested, pyridoxine was noted to have the best effect on root growth. Kodandaramaiah and Gopala Rao (1985) suggested that B-vitamins participated in plant growth and development by enhancing the endogenous levels of various growth factors (hormones) such as cytokinins and gibberellins. It may be thus concluded that luxuriant plant growth due to the exogenous application of WSF of rice polish and pyridoxine may help the plant to escape nematode attack or have a key role in the defence mechanisms of the plants to pathogens directly. Arrigoni *et al*. (1979) found lower concentrations of ascorbic acid (vitamin C) in susceptible tomato (*Lycopersicon esculentum* Mill.) cultivars than in resistant cultivars. They also reported that a decrease in plant ascorbic acid content can reduce the resistance, in tomato, to nematode infection. Melillo *et al*. (1983) showed that plants treated with ascorbic acid had fewer giant cells. Montasser (1990) reported ascorbic acid, nicotinic acid and riboflavin to be effective in reducing reproduction of *M. incognita* on tomato plants.

The plants treated with WSF of rice polish and pyridoxine solutions showed improved water absorption capacity of roots as compared to in the untreated control. The absorption of nutrients and water is closely associated with various physiological processes. B-vitamins are known to regulate various processes of metabolism. Their role in the absorption of nutrients has been studied by several workers. Dimitrova-Russeva and Lilove (1969) found an increased uptake of nitrogen and phosphorus by *Mentha piperita* due to the application of these vitamins. As has been discussed earlier, B-vitamins help plants to improve growth. Similar results were also obtained using B_6. The beneficial effects of B_6 in reducing the plant damage caused by the test nematodes simple, may prove an easy and cost-effective method of nematode management.

Conflict of interest: None.

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