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Integrated Approach for Management of Nematodes in Chickpea

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The efficient management of nematodes requires the carefully integrated combination of several methods. Each individual method of management has limited use, together; they help in reducing the nematode populations in agricultural soil or in plants more efficiently. Hence, the present study was carried out to determine the effect of mutagen (Hydrazine hydrate) treated seeds of chickpea var. 'GNG-469' with latex of *Jatropha panduraefolia* either alone or in combination against *Meloidogyne javanica* and *Rotylenchulus reniformis*. Statistical analysis showed that treated seeds significantly reduced nematodes population in soil and root galling and, improved plant growth parameters including chlorophyll content as compared to control. Severities of disease were shown more at 4000 J_2 inoculation of *M. javanica*. However, combined inoculations of *Meloidogyne javanica* and *Rotylenchulus reniformis* were also shown pathogenic severity as compared to separate inoculation. Therefore, plant growth improvement and disease severity are directly related with latex dressing of mutagen treated seeds and different inoculation rate of nematodes.

Key words: Nematodes, Mutagen, root galling, Management, Chickpea, Plant latex.

Chickpea (*Cicer arietinum* L.) is the premier pulse crop of Indian subcontinent. India is the largest chickpea producer as well as consumer in the world. The gap in demand and production continues to exist, and the country has to import chickpea every year to bridge this gap. Decline in production, a rising population, and small imports have caused the per capita availability of chickpea to fall over time. The plant-parasitic nematodes are widely distributed in most of the chickpea growing regions and are considered one of the important biotic constraints in the cultivation of chickpea. The overall effect of root-knot nematodes and reniform nematodes, which are important pests and occur in association with crop plants in the various regions of the world, is reduction in quality and quantity of crop yield (Adesiyan *et al*., 1990; Sharma & Mcdonald, 1990).

Damage caused by nematodes to plants is directly proportional to their population densities in soil, and their reproduction potential on the plants. The minimal density that causes a measurable reduction on plant growth or yield is regarded as the damage threshold density (Barker & Nasbaum, 1971), and when the cost of production and the value of given crop are considered, the term economic threshold density (Minimal nematode density that causes economic loss) is used. The threshold density varies with nematode species, race, plant variety and

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environment. Although a variety of chemical and other management tools are available to growers, none is perfect with respect to expense, environmental safety or efficacy against the changing nematode biotypes that often occur in agricultural soils. Thus, the main approaches to develop new control strategies are the lack of suitable methods and tools to investigate the effective management strategies against nematodes. Considerable information available in the literature has documented the effectiveness of latex bearing plants and various biopesticides in the management of plant parasitic nematodes (Ahmad *et al*., 2007; Ahmad & Siddiqui, 2009; Haggag, 2010). Many naturally occurring substances help to reduce cost over traditional chemical methods. Therefore, the objective of present research was to study the effective potential of mutagen treated seeds of chickpea var. GNG-469 either alone or with promising latex dressing of *Jatropha panduraefolia* Andr. against both *Meloidogyne javanica* and *Rotylenchulus reniformis* and to evaluate its effect on the growth of chickpea plant.

MATERIALS AND METHODS

Nematode culture

Reniform nematode

Soil samples were collected from six castor (*Ricinus communnis*) fields in Aligarh, India, in September 2008. Approximately 300 g soil was collected to a depth of 10-cm from each sampling site. The reniform nematodes were extracted from soil samples using Baermann funnel extraction method two nights before inoculation. Vermiform nematodes of *R. reniformis* were enumerated at 40X using an Olympus CK-2 inverted microscope, and utilized in glasshouse experiment.

Root-knot nematode

Second stage juveniles (J_2) of *M. javanica* were obtained from a pure culture that was previously initiated by egg masses and propagated on eggplant (*S. melongena*) in the glasshouse. Egg masses were hand picked using sterilized forceps from heavily infected roots. These egg masses were washed in distilled water, placed in 15 mesh sieves (8cm in diameter) containing crossed layers of tissue paper in Petri-dishes with water just deep enough to contact the egg masses and incubated 28 ± 2^0C to obtain freshly hatched second stage juveniles (J_2) for glasshouse experiment.

Glasshouse experiment

Seed treatment

Seeds of chickpea variety GNG-469 were pre-soaked in double distilled water for 6 hrs, prior to treatment with freshly prepared mutagen solution. The concentration (0.01%) of hydrazine hydrate (HZ) was prepared in phosphate buffer at pH-7. After the treatment, the seeds were dipped in 5% aqueous solution of sodium thiosulphate for 10 minutes and then washed thoroughly with running tap water. These mutagen treated seeds were further mixed with latex collected from *Jatropha panduraefolia* to give a uniform and smooth coating over the seeds. Seeds soaked in distilled water for 6 hrs were used as control. The treated as well as untreated seeds were sown in 15 cm diameter clay pots containing 1 kg sterilized soil-manure mixture. After emergence, the seedlings were thinned to one seeding per pot.

Nematodes inoculation and experimental design

Three week old plants were infested by pipetting 4000 both *Rotylenchulus reniformis* and *Meloidogyne javanica* suspensions either single or in combination into depressions (1.5-cm-diam. by 3- and 4-cm deep) surrounding the bases of the plants. Both species were at least 96% motile when applied to pots. The experimental design for each test was a complete randomized block with 4 replications. Untreated uninoculated plants served as control.

Observations

Plant growth characters

Plants were maintained for optimal growth by wetting as necessary every morning. At 70 days after inoculation, four replicates each were carefully lifted from the pots to avoid any damage to the roots. The roots were washed gently and thoroughly under running tap water. The different plant growth parameters including length in terms of centimeters (cm), fresh and dry weight in terms of grams (g) of shoots and roots were determined separately.

Chlorophyll content

Chlorophyll content of fresh leaf was extracted using the method of Hiscox and Israelstam (1979) by using dimethyl sulphoxide (DMSO) as an extraction medium, and estimated and calculated by the method of Arnon (1949). The

chlorophyll content was calculated according to the following equations-

$$\text{Chl a (mg g-1 FM)} = [(12.7 \times OD663) - (2.69 \times OD645)] \times \frac{V}{1000 \times W}$$

$$\text{Chl b (mg g-1 FM)} = [(22.9 \times OD645) - (4.68 \times OD663)] \times \frac{V}{1000 \times W}$$

where, V: volume of the extract; W: mass of the leaf tissue taken.

Pollen fertility

Fresh and young flowers from plants were taken from each treatment and the control. Pollen fertility was determined by staining the pollen grains with 2% acetocarmine solution. Pollen grains which took the stain and had a regular outline were considered as fertile, while the shrunken, empty and unstained ones as sterile. The following formula was used to calculate percent fertility and percent reduction (sterility):

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen}}{\text{Total number of pollen}} \times 100$$

$$\text{Percent reduction (Sterility)} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Nematode populations and root galls

The final population of *Rotylenchulus reniformis* in the pot soil was isolated with the help Cobb's sieving and decanting method along with Baermann's funnel technique (Southey, 1986). Infected root system of chickpea were washed with tap water, fixed in 4% formalin for 24 h and stained in 0.01 lactic acid fuchsine (Byrd *et al*., 1983) and examined for number of galls per root system.

Statistical analysis

Statistical analyses were performed using using SPSS 12.00 software (SPSS Inc., Chicago, IL, USA). Least significant differences (L.S.D) were calculated at $p = 0.05$ level to test for significant differences.

RESULTS

Data in Table 1 on the plant growth characters of chickpea revealed that single and combined inoculation levels of *M. javanica* and *R. reniformis* were found more pathogenic to host plant and showed maximum reduction in plant growth characters as compared to the control. It is interesting to observe that effect was more pronounced on roots than shoots of chickpea. However, the reduction due to concomitant inoculation was relatively less than the expected reduction caused by nematodes. A very significant improvement in length and dry weight of plant was maximum (55.4 cm & 11.6 g) after control in the treatment (mutagen+latex) at the 4000 inoculation of *R. reniformis* which were statistically superior to other treatments. Application of mutagen or latex alone was also almost equally effective in improving growth of plants with *M. javanica* and *R. reniformis*. Moreover, integration of mutagen treated seeds with latex of *J. panduraefolia* caused the greatest increase in plant growth characters in nematodes inoculated plants.

As a consequence of treatments, Chl *a* and Chl *b* content increased in fresh leaf with the inoculation of nematodes either alone or in combination. The reduction in chlorophyll content was found maximum in untreated inoculated control with combined inoculation of nematodes (Table 1). However, application of both treatments (mutagen+latexat) at 4000 inoculation dose of nematodes either alone or in combination had significant improvement in chlorophyll content compared to their respective control. Per cent reduction in pollen fertility was also found minimum in combined application of both treatments (mutagen treated seeds with latex) in the stress of nematodes either alone or in combination. The latex treatment had slightly more potential for improvement of plant growth characters and reduction in root galling, and nematode soil population as compared to mutagen treatments. Treatments with mutagen and latex significantly reduced galling and nematode population over the control (Table 1). In alone treatments, reductions in root galling and nematode population in soil were also found significantly. So far as treatment is concerned, there was always an increase in growth parameters with alone or combined application of treatments, and both treatments were effective enough in managing nematodes. The reductions in different parameters due to nematodes were also found to have positive correlation with root diseases caused by nematode multiplication, thus indicating a pathogenic effect of the nematodes. It was also observed that both nematodes were mutually inhibitory to each other in concomitant inoculations (Table 1).

Table 1. Effect of latex dressing of mutagen treated seeds of chickpea variety GNG-469 on nematode multiplication, root-knot development and plant growth

Treatment	Nematodes		Plant length			Plant fresh weight			Plant dry weight			Pollen fertility		Chlorophyll content (mg/g)		Root galls/ plant	Roty/ pot
	Mel.	Rot.	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Actual%	reduction	Chl. a	Chl. b		
UU	-	-	46.2	23.4	69.6	35.4	32.0	67.4	9.0	6.3	15.3	90.0	-	1.6	1.0	-	-
UI	4000	-	31.4	13.3	44.7	18.7	12.5	31.2	4.2	1.9	6.1	48.2	46.4	1.1	0.7	160	-
	-	4000	33.0	15.1	48.1	19.7	14.3	34.0	4.8	3.0	7.8	51.0	43.3	1.2	0.8	-	22112
	4000	4000	23.7	10.2	33.9	11.4	7.2	18.6	2.9	1.0	3.9	44.9	50.1	1.0	0.6	107	13107
Mutagen	4000	-	33.5	15.2	48.7	20.2	14.0	34.2	50.3	2.7	8.0	78.3	13.0	1.2	0.8	147	-
	-	4000	34.9	15.9	50.8	22.1	15.8	37.9	5.6	4.2	9.8	83.0	7.8	1.3	0.9	-	20690
	4000	4000	26.5	11.6	38.1	13.7	10.2	23.9	3.3	1.8	5.1	74.0	17.8	1.1	0.7	90	12182
Latex	4000	-	35.2	16.4	51.6	22.1	16.4	38.5	6.0	2.9	8.9	80.0	11.1	1.4	1.0	132	-
	-	4000	36.1	17.0	53.1	24.3	17.6	41.9	6.2	4.4	10.6	84.8	5.8	1.4	1.1	-	18025
	4000	4000	27.0	11.9	38.9	20.1	10.5	30.6	3.5	1.9	5.4	76.4	15.1	1.2	0.9	82	11040
Mutagen+Latex	4000	-	37.5	17.1	54.6	23.3	17.3	40.6	6.5	3.1	9.6	81.6	9.3	1.5	1.1	118	-
	-	4000	38.0	17.4	55.4	25.9	18.7	44.6	6.9	4.7	11.6	86.0	4.4	1.7	1.2	-	15034
	4000	4000	28.2	12.2	40.4	22.6	12.0	34.6	4.6	2.0	6.6	78.1	13.2	1.2	1.0	70	10230
C.D *(P = 0.05)*	1.03	0.61	1.69	1.14	0.99	2.14	0.31	0.07	0.49	3.62	-	0.21	0.10	7.83	756.24		

Mel. = *Meloidogyne javanica; Roty.* = *Rotylenchulus reniformis;* Each mean consists of four replications.

DISCUSSION

The suppressive effects of some plant parts/products on nematode populations have been well documented in several pathosystems (Rather 2008; Ahmad, 2009). These plant materials could be developed for use as nematicides themselves, or could serve as model plant materials for the development of environment friendly nematicides. This study was designed to evaluate the nematicidal activity of the latex of *J. panduraefolia* with mutagen treated seeds on the nematodes. In glasshouse experiments clearly demonstrated that survival and reproduction rate of the nematode were significantly reduced by the both applied treatments (mutagen & latex). Kundi *et al*., (1997) reported differential sensitivity within crop and even genotype. The sensitivity depends upon its genetic architecture and the mutagens employed (Blixit, 1970) besides, the amount of DNA, its replication time in the initial stages and degree of heterochromatin. These criteria are responsible for differential mutagenic sensitivity in a crop. In this study, a result revealed that the reduction in pathogenic activity of nematodes might be possible either due to mutagenic sensivity in chickpea crop towards nematodes feeding or impairs nematode neuromuscular activity by inhibiting the function of the enzymes resulting in reduced movement and ability of invasion and multiplication.

The data for the improvement activity in plant growth characters of chickpea with the applications of latex agree with results of other researchers, who found that the population density of nematodes was reduced when host plant seeds were treated with latex of various plants (Siddiqui & Alam, 1988abc). The principle of nematode management by the latex can be attributed to the unfavorable conditions in latex dressing of seeds for both *M. javanica* and *R. reniformis* which might have subjected to lesser penetration and later retardation in biological activities of nematodes like feeding or breaking and lengthening the life cycle of nematode. As a result of application of latex dressing, plant nutrients are released which accelerates root development and overall plant growth, thereby helping the plants to escape nematode attack and growth of chickpea.

Plant nematodes control and management have remained a crucial issue throughout the world. Several well known methods and strategies have been adapted in developed and developing agriculture. Adesiyan *et al*., (1990) emphasized that the success and adoption of such methods depend largely on the level of expertise and socio-economic situation of farmers. Seed dressings with latex brought about a significant reduction in multiplication of test nematodes with a corresponding increase in plant growth characters. Plant latex researches strongly indicate the presence of some factors which are active against nematodes even in small quantity. Latices are rich in many biologically active chemicals such as alkaloids etc (Siddiqui & Alam, 1990). Interesting results were reported by Siddiqui & Alam (1988abc) in series of papers regarding the systematic activity of plant latices against nematodes on some vegetables. These plant latices used as seed dressing treatment inhibited the development of nematodes and consequently improved plant growth. This study has confirmed the potential of latex dressing of mutagen treated seeds, which when used in experiment is capable of managing pathogenic activity of nematodes. This is understandable since latex dressing of mutagen treated seeds is comprised of pool of biologically active methods which need to be further researched for better utilization.

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