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EFFICACY OF BOTANICALS AND CARBOFURAN FOR THE CONTROL OF *MELOIDOGYNE INCOGNITA* AFFECTING *SOLANUM LYCOPERSICUM* L.

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A B S T R A C T

Fresh leaves of *Calotropis procera*, *Wedelia chinensis*, *Lantana camara, Jatropha pandurifolia, Parthenium hysterophorus* and *Nerium indicum* were evaluated for their hatching and mortality of *Meloidogyne incognita.* After that tested plants were used as an organic amendment for the management of *M. incognita* affecting Tomato. Four concentrations S, S/10, S/100 and S/1000 of leaf extracts of tested plants were prepared. All the plant extracts showed varied and significant results over control. The "S" concentration of leaf extracts of *C. procera, P. hysterophorus, L. camara* and *W. chinensis* exhibited 100% mortality over *J. Pandurifolia, N. indicum* after 48 h exposure period. Similarly, larval hatching was completely inhibited by "S" concentration of extracts of tested plants except for *N. indicum* after five days of the exposure period. Increased dilution showed a direct effect on hatching but a contrary effect on mortality. The juvenile mortality increased with increase in exposure period. In pot condition, soil amended with chopped leaves of tested plants 50 g and100 g alone and in combination with 50 g leaves plus carbofuran significantly enhanced the plant growth and decreased host infestation caused by *M. incognita* over control. Among treatments, *C. procera* leaves plus carbofuran were found to be the best in increasing plant growth and chlorophyll content.

Keywords: Botanicals, hatching, mortality, root-knot nematode, organic amendment, nematicide.

INTRODUCTION

Plant-parasitic nematodes are recognized as potentially serious threat to crop productivity. These are now a day's most widespread pathogen limiting world agricultural productivity. Among the plant parasitic nematodes, most economically damaging genera is the root knot nematode, *Meloidogyne* species. Root-knot nematodes, *Meloidogyne* spp. are found that serious threat on horticultural and field crops causing an estimated loss of US$100 billion globally on an annual basis (Khan *et al.*, 2008). In India, root-knot nematode *M. incognita* affects vegetable crops and infestation measured up to 85% on tomato, eggplant, okra, cucurbits, potato, papaya, jute, cotton and groundnut etc. The estimated loss due to *M. incognita* is 46.2%, 99% and 27.3% in tomato, okra and brinjal respectively (Akhtar, 1999; Netscher and Sikora, 1990). It is desirable that to control this threat of crop productivity and yield. For the management chemical and non-chemical methods have been undertaken to control the root-knot nematode. They include the use of nematicides, organic amendments, resistance cultivars, soil solarization and biological control which have been found to be effective at a different level for the protection of tomato plants (Sakhuja and Jain, 2001). Among them chemical nematicides are found to be most desirable, easy to apply and show rapid effects, they have begun withdrawing from the market because of public health and environmental safety (Rich *et al.*, 2004). So, from this view non-chemical methods have been preferred because these are less toxic and significantly controlled the root-knot nematodes. Higher plants are the reservoirs of many compounds and secondary metabolites present in the leaf extracts having nematicidal properties against *M. incognita* (Pavaraj *et al.*, 2012). The use of organic amendment is preferred for the control of root-knot nematode because organic amendments are less toxic, less hazardous and pollution free. In the present study, an attempt has been made to determine the effect of some botanicals as organic amendments known for their nematicidal properties and to integrate them with a

nematicide viz., Carbofuran/Furadan to have a maximum reduction of root-knot nematode *M. incognita* affecting tomato. There is need to develop alternative methods for control of root-knot nematode that are cheap, environmentally friendly and not harmful to humans. These botanicals not only control nematodes but also improve the soil productivity and crop yield by several folds. The use of botanical extracts for controlling the root knot nematodes is becoming more appealing because of the growing problems of environmental pollution arising from the use of the persistent pesticide.

MATERIAL AND METHODS

Preparation of nematode inoculums: Egg masses were handpicked using sterilized forceps from infected tomato roots. The egg masses were washed in distilled water and placed in 15 mesh sieves (9 cm in diameter) containing crossed layers of tissue paper. The sieve was placed in a Petri-plate containing distilled water deep enough to contact the egg masses. A number of these assemblies were kept in an incubator running at 25± 1°C in order to obtain the required number of second-stage juveniles for inoculation. The infective second stage of juvenile of *M. incognita* was adjusted so that 1000 freshly hatched juvenile (J2) could be added in 10 ml of water suspension and used for the experiment.

Preparation of plant extracts: Leaves of six different plants viz., *Parthenium hysterophorus, Calotropis procera*, *Wedelia chinensis, Nerium indicum, Jatropha pandurifolia* and *Lantana camara* were thoroughly washed, chopped and 50 g of each macerated in a grinder using distilled water and make up the volume 150 ml kept for 24 hours. These were then filtered through Whatman filter paper no.1 and extract arbitrary termed as standard 'S'. Other dilutions viz. S/10, S/100 and S/1000 were prepared from the standard 'S' concentration with distilled water.

Hatching experiment: For hatching experiment, 5 egg masses were taken from thoroughly washed roots of tomato infected with root-knot nematode *M. incognita*. Egg masses of average size were kept in 40 mm Petri-plates containing 10 ml of leaf extract of each dilution (S, S/10, S/100 and S/1000) separately. Petri-plates containing distilled water served as control. There were three replicates of each treatment. The total numbers of the hatched larvae were counted after 5 days, with the help of counting dish under a stereoscopic microscope.

Mortality experiment: For mortality study 10 ml of a water suspension containing 1000 second stage larvae of *M. incognita* were poured in different concentrations (S, S/10, S/100 and S/1000) of leaf extracts separately. There were three replicates of each treatment. The Petri-plates were kept at 28°C. Numbers of immobile larvae were counted after 12, 24 and 48 hours of the exposure period. The death of the larvae was ascertained by transferring the immobilized larvae into the water for 1 hour and percent mortality was calculated.

Organic soil amendment: Clay pots (15 cm diameter) filled with 1 kg autoclaved soil was amended with chopped leaves of different tested plants at 50, 100 g alone and with 50 g+ nematicides (1 g carbofuran) in the mixture. The pots were watered immediately after the treatment and left for one week for proper decomposition of organic additives. Three week old seedlings of tomato cv. K- '21' were transplanted into pots after waiting period of one week. After seven days, these plants were inoculated with 1000 freshly hatched second stage juveniles (J_2) of *M. incognita* by making 3.5 cm deep, 4-5 holes in a radius of 1.5 cm in the rhizosphere of the plant. The holes were then plugged gently with soil. The untreated inoculated/untreated un-inoculated pots served as control.

Chlorophyll content: The chlorophyll in the fresh leaf was estimated following the method (Mackinney, 1941). One gram fresh leaves were ground to a fine pulp using a mortar and pestle after pouring 20 cm^3 of 80% acetone. The mixture was centrifuged at 5,000 rpm for 5 minutes. The supernatant was collected in 100 cm^3 volumetric flasks. The residue was washed three times, using 80% acetone. Each washing was collected in the same volumetric flask and volume was made up to mark, using 80% acetone. The absorbance was read at 645 and 663 nm for chlorophyll against the blank (80% acetone) on a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

Root-knot index: The root-knot index (RKI) was determined following the method (Heald *et al*., 1989). Root samples were rated for root-knot nematode infection by a gall rating of 1-5 scale, where 1= no galls, 2 = 1-25%, 3 = 26-50%, 4 = 51-75%, and 5 = > 75% of the roots galled.

Randomized block design: The experiment was carried out in a randomized block design and experiment was divided into nineteen sets. 1. Control (inoculated), 2. Cp50- *C. procera* leaves at 50 g/pot 3. Cp100- *C. procera* leaves at 100 g/pot 4. Cp50+N- *C. procera* leaves at 50 g+1 g carbofuran/pot 5. Jp50- *J. pandurifolia leaves* at 50 g/pot 6. Jp100- *J. pandurifolia leaves* at 100 g/pot 7. Jp50+N- *J. pandurifolia leaves* at 50 g+1 g carbofuran/pot 8. Wc50-

W. chinensis leaves at 50 g/pot 9. Wc100- *W. chinensis* leaves at 100 g/pot 10. Wc50+N- *W. chinensis* leaves at 50 g+1 g carbofuran/pot 11. Ph50- *P. hysterophorus* leaves at 50 g/pot 12. Ph100- *P. hysterophorus* leaves at 100 g/pot 13. Ph50+N- *P. hysterophorus* leaves at 50 g+1 g carbofuran/pot 14. Lc50- *L. camara* leaves at 50 g/pot 15. Lc100- *L. camara* leaves at 100 g/pot 16. Lc50+N- *L. camara* leaves at 50 g+1 g carbofuran/pot 17. Ni50- *N. indicum* leaves at 50 g/pot 18. Ni100- *N. indicum* leaves at 100 g/pot 19. Ni50+N- *N. indicum* leaves at 50 g+1 g carbofuran/pot**.** Each treatment was replicated 3 times, 19×3= 57 pots and pots were arranged in a randomized design.

Observations: The plants were uprooted after 90 days of inoculation, washed thoroughly and different plant growth parameters like shoot length (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), the number of fruits, chlorophyll content (mg/g fresh mass) and root-knot index (RKI) were determined.

Statistical analysis: The data were analysed by one-way analysis of variance (ANOVA) using R software (R Development Core Team 2011). The least significant difference (L.S.D) were calculated at P=0.05 to test for significant differences.

RESULTS

The results presented (Figure 1) that water extracts of all the tested botanicals have the deleterious effect on the larval hatching of *M. incognita* after 5 days. But hatching gradually decreased from lower concentration to higher concentration. Aqueous dilutions of leaf extracts of different plants were inhibited larval hatching of *M. incognita* to a varying degree. Highest inhibition of larval hatching of *M. incognita* was noticed in *C. procera* in different concentrations viz. S, S/10, S/100, and S/1000 followed by *L. camara, W. chinensis, P. hysterophorus, J. pandurifolia,* whereas lowest inhibition on larval hatching was recorded in *N. indicum* over control (distilled water).

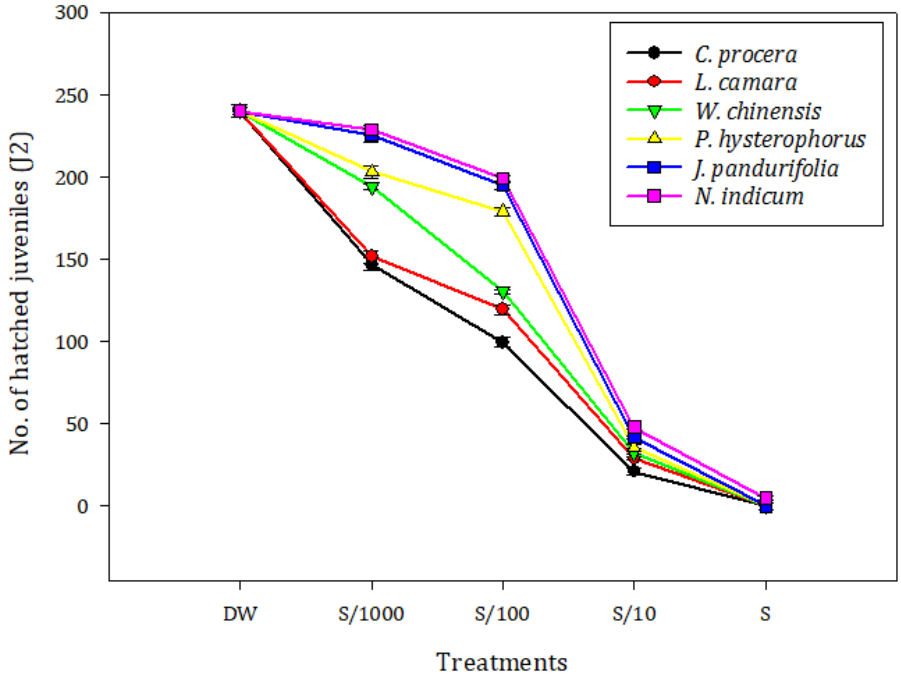


Figure 1. Effect of water extracts of different plants on the egg hatching of *Meloidogyne incognita* in-vitro (after 5 days). DW = Distilled water (control). S=Standard Concentration, S/10, S/100 and S/1000 are dilutions of 'S'; Each bar represents treatment mean of three replicates ± SE.

All the leaf extracts of different plant species were tested on the mortality of *M. incognita* juveniles. The nematicidal effect on the mortality of root-knot nematode larvae differed with different concentrations of leaf extracts and the duration period (Figure 2 a-f). Among leaf extracts *C. procera* proved highly toxic to larvae was found in S concentration followed by *J. pandurifolia, W. chinensis, P. hysterophorus*, *L. camara* and *N. indicum* respectively. The percent mortality of *M. incognita* juveniles varied in different dilutions of leaf extracts such as S, S/10, S/100 and S/1000 over control (distilled water) after 12 h of the exposure period.

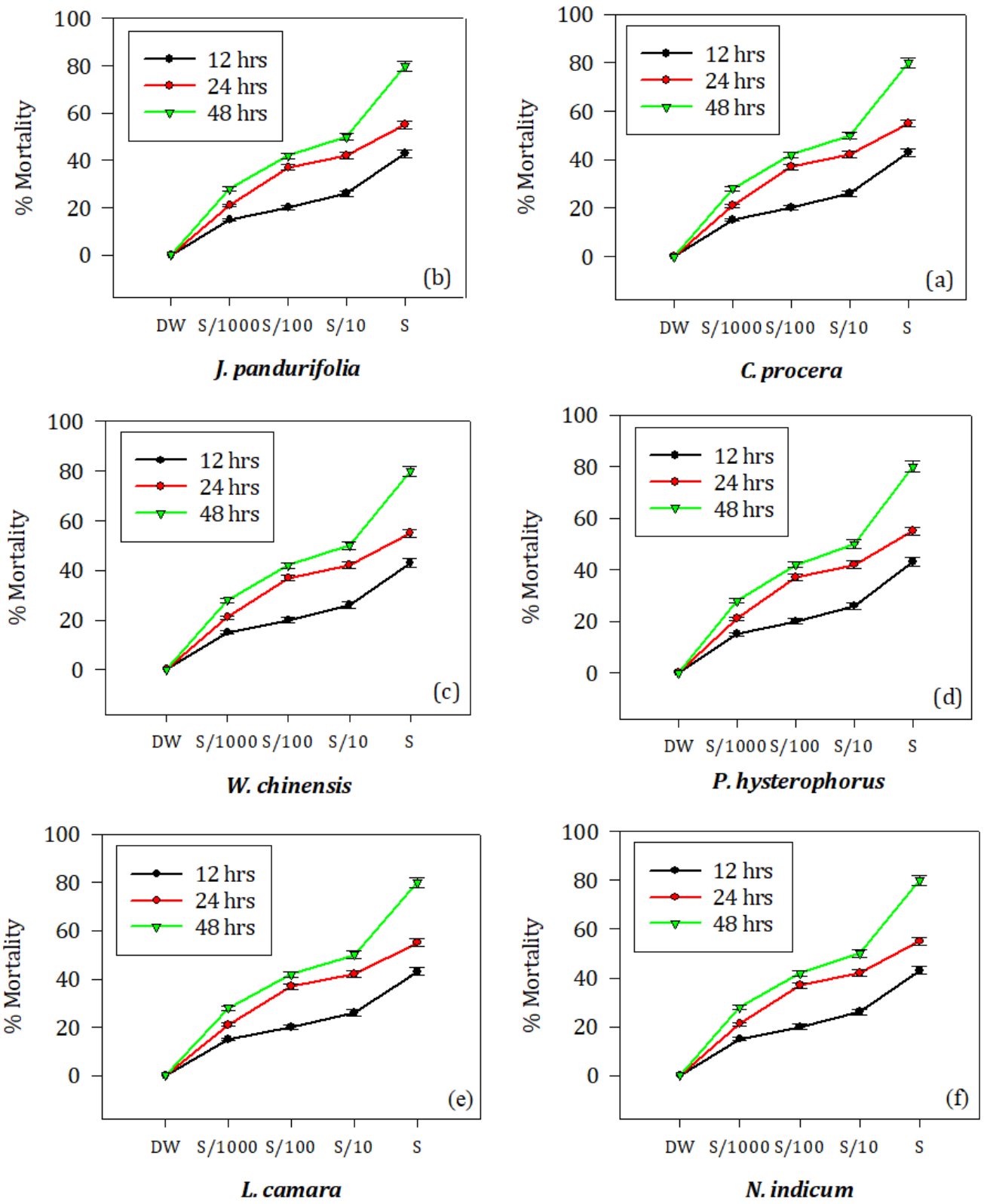


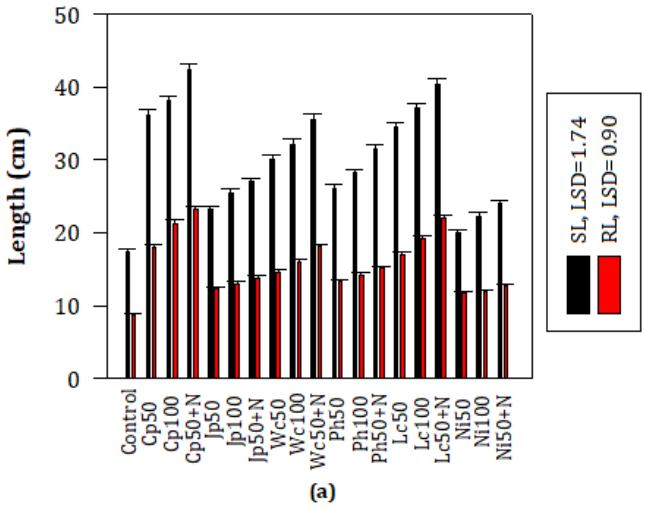
Figure 2. (a-f). Effect of water extracts of different plants on the mortality of Meloidogyne incognita in vitro (after 12, 24, 48 hours). DW= Distilled water (control) S= Standard Concentration, S/10, S/100 and S/1000 are dilutions of 'S'; Each bar represents treatment mean of three replicates ± SE.

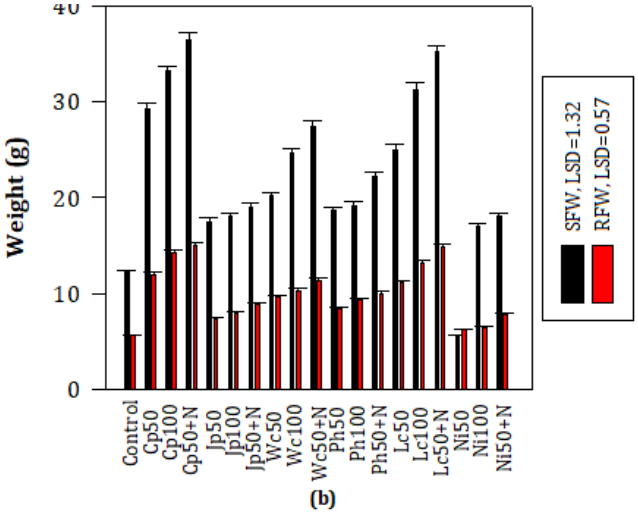
All the plant extracts examined showed the nematicidal effect to a varying degree with the leaf extracts of *C. procera*, *J. pandurifolia, W. chinensis, P. hysterophorus*, *L. camara* and *N. indicum* respectively showing percent mortality in "S" concentration after 24 h exposure period onwards.

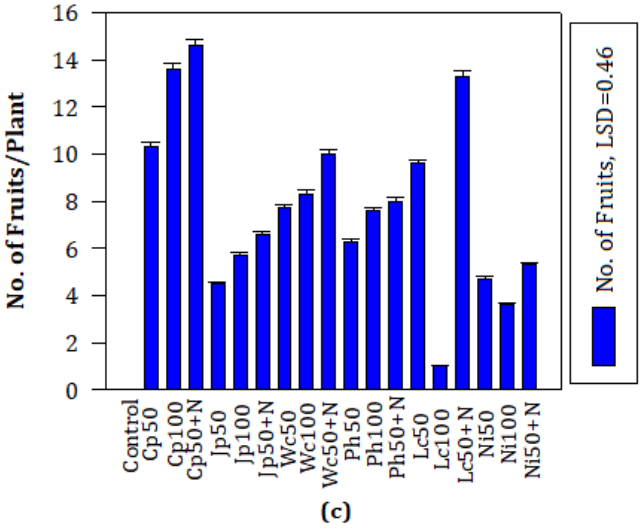
Among all the tested plant extracts *C. procera*, *W. chinensis, P. hysterophorus*, *L. camara* was the only extract showing 100% mortality in "S" after 48 h of the exposure period. The results show that the "S" concentrations of leaf extracts of *J. pandurifolia,* and *N. indicum* were at par in causing mortality of J2 after 48 h exposure period onwards (Figure. 2 b and f). *N. indicum* leaf extract proved to be significant but least effective in causing mortality among all the tested plants. All the results show that the time of exposure plays a prominent role in enhancing mortality of Juveniles. As the time of exposure was extended from 12 to 48 h, the mortality rate increased in all concentrations. From the results, it can be inferred that concentration of plant extracts, as well as exposure time, have a direct effect on the mortality of *M. incognita* juveniles.

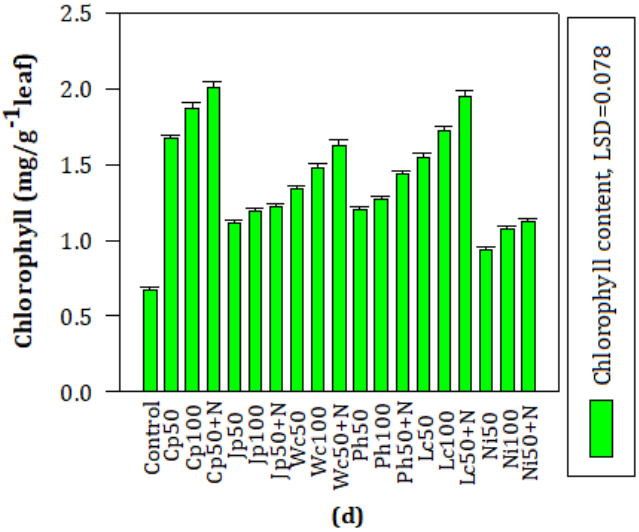
Due to greater nematicidal activity shown by all the tested botanicals under laboratory condition, they were selected for testing their ability to control root-knot nematode *M. incognita* under pot conditions.

Data in (Figure: 3 a-e) show a significant improvement in plant growth, chlorophyll content and reduction in the host infestation caused by *M. incognita* to the root-knot nematode due to soil application of botanicals and carbofuran over control plants. The improvement in growth and chlorophyll content was more pronounced by the application *C. procera* 50 g /pot with 1 g carbofuran (nematicide) followed by 100 g/pot and 50 g/pot leaves alone. (Figure: 3a-e).









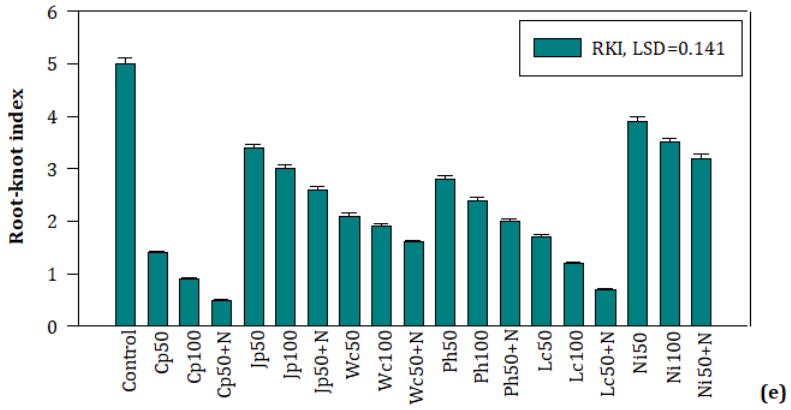


Figure 3 (a-e). Effect of leaves of different plants as organic amendment alone and with carbofuran (nematicide) on the growth, chlorophyll content and root-knot index of *S. lycopersicum.* (SL- Shoot length, RL- Root length, SFW- Shoot fresh weight, RFW- Root fresh weight). (Cp= *Calotropis procera* Jp= *Jatropha pandurifolia* Wc= *Wedelia chinensis* Ph= *Parthenium hysterophorus* Lc= *Lantana camara* Ni= *Nerium indicum* and N= Nematicide (Carbofuran). Each bar represents treatment mean of three replicates ± SE.

Other plants like *N. indicum* and *W. Chinensis* leaves also showed significant improvement in plant growth and chlorophyll content over untreated inoculated control. Application of *N. indicum* leaves though significantly improved the growth and chlorophyll content over control but comparatively, it was the least effective among all the treatments. The results presented (Figure: 3 e) show that there was a reduction in root-knot disease incidence due to the soil application of leaves of all the tested plants.

C. procera leaves at 50 g with 1 g carbofuran/pot were caused maximum reduction in a number of galls/root system followed by *J. pandurifolia, W. chinensis, P. hysterophorus* and *L. camara, N. indicum* leaves at 100 g and 50 g /pot alone. Among all the treatments, *C. procera* though significantly showed a reduction in nematode index of tomato over inoculated control. The variations in nematicidal action of the tested plants may be attributed to the differences in the nature of metabolites produced during decomposition of the organic matter.

DISCUSSION

Plant parasitic nematodes were caused serious problems to the cultivated crops (Sasser and Freckman, 1987). These were not only suppressing plant growth but also interfere in the nodulation, nitrogen fixation and adversely affect the overall yield. During in vitro study leaf extracts of *C. procera* was found highly deleterious to *M. incognita* juveniles (Singh *et al.*, 2001). This is due to metabolites present in the plants as a potential source of nematicidal compounds. Many of them have been found in plants as a source of alkaloids, diterpenes, fatty acid, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls, which are generally safe for the environment and humans (Chitwood, 2002). After that effect of chopped leaves of all tested plants with a combination of nematicide in pot condition also found highly efficacious against root-knot nematode *M. incognita* affecting tomato. It was concluded that all the tested plants have nematicidal properties (Sosamma and Jayasree, 2002). Moreover, the nemato-toxicity due to the presence of some chemicals in the extracts of plants cannot be ruled out. All the treatments significantly reduced the root-knot index caused by *M. incognita.* Among the tested plants against the root-knot nematode, *C. procera* was found most effective as an organic amendment (Ramakrishnan *et al.*, 1997). The highest reduction in root-knot development was found in amended with *C. procera* leaves with carbofuran followed by an increase in growth tomato plants. Nematicides are generally recommended for the control of root-knot nematodes and targeted by 48% of globally use across crops (Coyne *et al.*, 2009) but these are costly and produce environmental hazards (Zureen and Khan, 1984). The high cost of nematicides were not afforded by farmers and to overcome this condition, new alternative strategies was undertaken to control root-knot nematode population and disease intensity. This may be partly due to the reduction in root-knot development and partly due to fact that organic additives also served as manures. Thus, it can be concluded after the thorough study of

above mention observation that chopped leaves alone and in combination with carbofuran could be of great use in solving of some of the nematode problems. As these phyto-chemicals are easily available in wild condition and do not harm the environment. Hence, the present study has been carried out to evaluate plant parts for their nematicidal properties against root-knot nematode *M. incognita.*

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