

SUMMARY

Medicinal plants are high valued remunerative crop earning foreign exchange. These are used as raw material in flavouring pharmaceutical, perfumery and cosmetic industries of the world. *Ocimum Sanctum*—renowned medicinal plant belonging to family Lamiaceae, commonly known as Tulsi, is grown in almost all parts of India and is held sacred by Hindus all over the country. It is also grown as an ornamental plant. A large number of the plant parasitic nematodes have been reported in the rhizosphere of different medicinal plants affecting the quality and quantity of the produce and the economy of the crop in many ways. The root-knot nematode *Meloidogyne incognita* causes considerable losses to a number of plants including medicinal and ornamental plants. The following studies were carried out on *O. sanctum* in order to observe the effects of *M. incognita* on plant growth, yield and some biochemical parameters; and to examine the effects of fly ash amended soil on the plant growth and development of root-knot nematode, *M. incognita*.

Meloidogyne incognita, soon after penetration, induced giant cell formation, hypertrophy and hyperplasia around the giant cells in the roots of *O. sanctum*. These changes led to the formation of prominent galls. As a result of infection, the plants exhibited stunting and loss of weight. The ultimate result of infection on root anatomy and physiological activities of the host plant was yield loss.

In the first experiment, *M. incognita* infected roots were examined from the day one to the 27th day of inoculation. The

anatomical studies were carried out at regular intervals of time in order to investigate sequential changes in the formation of giant cells, in the development of the nematode, in the formation of hypertrophic and hyperplastic tissue, and development of abnormal vascular elements.

The second-stage juveniles of *M. incognita* penetrated the young root tips of *Ocimum sanctum* within 24h of inoculation. Soon after penetration they migrated towards the zone of cell elongation and the zone of cell differentiation. The juveniles while migrating towards the zone of elongation caused hypertrophy and hyperplasia which was established when enlarged cells with enlarged nuclei were observed near the body of the juvenile. Enlarged cells or in other words incipient giant cells enclosing single large nucleus with large nucleolus were observed after 48h. The developing giant cells having two or more nuclei were also observed. Their shapes changed from elongated to globular. Their cell walls became very thick. The root swelled up assuming the shape of gall after six days of inoculation. The size of giant cells, as well as the size of nucleus and nucleolus, also increased. The cytoplasm of giant cell became extremely dense and granular after nine days of inoculation. The shape of nuclei varied considerably such as globose, ovoid, elongated, triangular and even amoeboid. After 15 days of inoculation, the giant cells attained their largest size. The size of nuclei increased enormously. In some giant cells the nuclei were found arranged in the form of a cluster. The giant cells appeared to be surrounded by xylem comprising of vessel elements of different shapes and sizes. The nematodes after 27 days of inoculation, developed into the form of mature females.

Some mature females also started egg laying. Some giant cells were devoid of cytoplasm.

As far as abnormalities in vascular elements are concerned, the differentiation of vessel elements was observed near the developing giant cells, after 48h of inoculation. Distortion of vessel elements occurred after six days of inoculation. Hypertrophic and hyperplastic tissues were also observed near the giant cells after six days of inoculation. After nine days of inoculation, the cells near abnormal vessel elements exhibited hypertrophy and hyperplasia. The giant cells were found connected with a branch of vascular strand after 15 days of inoculation. After 21 days of inoculation, vessel elements of different shapes and sizes were found near the giant cells. The abnormal vessel elements adjacent to the giant cells were very large and their shapes resembled with the shapes of the giant cells. Abnormal vessel elements with thick reticulate cell walls were observed after 27 days of inoculation. From these observations, it was hypothesized that the formation of abnormal vessel elements in excessive amount in affected part probably retained water in large amount, or diverted water supply towards the giant cells, or acted as mechanical tissue to provide protection to the giant cell, or provided support to the entire gall to prevent it from collapsing.

From our study it might be concluded that the giant cells that appeared completely surrounded by abnormal xylem elements were not actually completely enveloped but were connected with the phloem. The phloem region appeared to be the only preferential feeding site of the nematodes. After the

development of the giant cell, the sieve tube elements, in the secondary phloem appeared to be diverted towards the giant cells, when seen in transverse section. In this way, the supply of assimilates to the giant cells was not disrupted. The giant cells, thus obtain metabolites continuously through phloem elements.

The second experiment was performed to ascertain the effects of different inoculum levels of the nematode on the growth and yield of the plants, on the formation of galls, on the development of the nematode and on the formation of abnormal tissues in the galls.

The host plants responded differently to different densities of initial population. There are reports that low population of the nematodes may be harmful or beneficial or may not affect plant growth. The plants of *O. sanctum* responded differently to different population densities. At the lowest inoculum level ($P_i=5 J_2$), slight but non-significant decrease was observed in comparison to control. At higher inoculum levels the growth decreased significantly. The reduction in all growth as well as yield parameters was maximum at 5,000 J_2 , the highest inoculum level.

The galls were scanty and very small at lowest ($P_i=5 J_2$) initial inoculum level. The gall number and the gall size increased from lower to higher inoculum levels with the maximum at the highest inoculum level. The number of mature females recorded from plants at $P_i=5J_2$ increased to maximum at $P_i=5,000 J_2$.

At lowest inoculum level one nematode was enough to cause the formation of giant cell complex, while at higher

inoculum levels more nematodes were found causing multiple giant cell complexes. There were great variations in the size of the giant cells. At lower inoculum levels, the nematodes and the giant cells were found at one or two places, when seen in transverse section. At higher inoculum levels, all the four parenchymatous rays were seen occupied by the nematode and the giant cell. The giant cell cytoplasm was more dense at lower inoculum level than at higher inoculum level. The amount of abnormal xylem was higher at higher inoculum levels than at lower inoculum levels. Similarly, the phloem strands were also less distorted at lower than at higher inoculum levels. Phloem elements were found associated with every giant cell cluster.

The third experiment was designed to observe the effects of different inoculum levels of the nematode on growth, chlorophyll pigments and oil contents of the leaves of *Ocimum sanctum*. The growth parameters, amount of chlorophyll pigment, and oil content decreased in all the plants inoculated with the nematode. The reductions were higher at higher inoculum levels.

The fourth experiment was conducted to investigate the effect of soil application of fly ash, in different concentrations on plant growth, yield, leaf chlorophyll pigmentation and oil content of *Ocimum sanctum*. In the first year of experiment an uphill trend in plant length, weight (fresh as well as dry), seed yield, amount of chlorophyll a,b and total chlorophyll and oil content was observed at 10% fly ash level to 30% level, whereas at the fly ash levels higher than 30%, a downhill trend, in same parameters was encountered when comparisons were made with the control. The second year experiment also followed the same trend with slight

differences. However, in the third year experiments reductions in all parameters, in comparison to control, were observed. This finding led to the conclusion that fly ash amendment did not favour the plant growth as it altered various characteristics of the soil, which in turn produce marked effects on the plants.

In the fifth and the last experiment, the effects of fly ash and the root-knot nematode, *Meloidogyne incognita*, on *O. sanctum* were ascertained. In all the three years of experiment fly ash amendment caused increase in growth and other parameters of plants inoculated with *M. incognita* when compared with inoculated control plants grown in unamended soil. The increase was more pronounced in first year followed by second year. Fly ash, at varying concentration, influenced the nematode differently. Root galling, egg mass production and even morphometrics of the nematodes was affected adversely at higher fly ash level. The reductions in the number of galls per plant, number of egg masses per plant, length and width of mature female, length and width of neck and stylet became more pronounced in second and third years of experiment.