

Introduction

INTRODUCTION

Herbal medicines have been in use since time immemorial and are still a vital component of our national and medical heritage. For a long time, herbs have been prized for their pain relieving and healing attributes, and even today we still rely on the curative properties of plants in about 75% of our medicines. It has been estimated by WHO that 80% of the earth's six billion inhabitants rely upon the traditional medicines. Therefore, medicinal plants occupy an integral position in our socio-cultural lives. Over the centuries, societies around the world have developed their own traditions to make sense of medicinal plants and their uses. Some of these traditions and medicinal practices may seem strange and magical, others appear rational and sensible but as a matter of fact these attempts are to overcome illnesses and sufferings, and to enhance the quality of life. Yet despite the dramatic advances and advantages of conventional medicines, or biomedicines as these are also known, it is clear that herbal medicines have much to offer. We tend to forget that in all but the last fifty years or so, humans have relied almost entirely on plants to treat all manner of illnesses, from minor problems such as cough and cold to life threatening diseases such as tuberculosis and malaria.

The increasing trend of awareness towards the utility of medicinal plants has necessitated compilation of recent developments in this field. The variety and sheer number of plants with therapeutic properties are quite astonishing. It has

been assessed that around 70,000 plant species, ranging from lichens to towering trees, had been used at one time or the other for medicinal purposes. Today, the manufacturers of herbal medicines in the advanced western countries are still making use of at least a thousand indigenous European plants as well as several thousand species native to America, Australia and Africa. In Ayurveda (an Indian traditional system of medicine) about 2,000 plant species are considered to have medicinal properties, while the Chinese pharmacopoeia lists over 5,700 traditional medicines, most of which are of plant origin.

The genus *Ocimum* is an aromatic herbaceous plant used in perfumery, flavouring and pharmaceutical products (Khosla, 1995). Several species of *Ocimum* like. *O. basilicum* Benth, *O. canum* Sims, *O. gratissimum* L., *O. kilimandscharicum* Guerka, *O. sanctum* L. are among some of the highly priced economically important medicinal and aromatic plants (Haseeb *et al.*, 1998). *Ocimum sanctum*, commonly referred as Tulsi (meaning matchless), has some unique medicinal properties. It is an erect, herbaceous, much branched, pubescent, annual, 30–75 cm tall plant. It belongs to the family Labiatae (Lamiaceae) and is found throughout India from Andaman and Nicobar islands to Himalyas upto 1,800m altitude. The leaves are elliptic oblong, acute or obtuse, entire or serrate, pubescent on both sides, minutely gland dotted. The flowers are purplish or crimson, arranged in verticillasters. The nutlets are sub-globose or broadly ellipsoid, slightly compressed, nearly smooth, pale brown or reddish, with small black markings.

In Ayurveda (The indigenous system of medicine in India is known as Ayurveda [Ayu means life; veda means knowledge] which is the science of living and longevity), *O. sanctum* is considered to have a wide range of uses such as in relieving fever, bronchitis, asthma, stress and mouth ulcers. Different parts of *O. sanctum* have been used in folk medicine as an anti-inflammatory remedy for the treatment of acute and chronic inflammations (Singh *et al.*, 1995). *O. sanctum* is commonly grown in gardens and is frequently found as an escape. The plant is held sacred by Hindus all over India and is frequently propagated in their courtyards, kitchen gardens and in the temples. At least two varieties of *O. sanctum* are met with in cultivation: the Sri tulsi with green leaves, which is the most commonly grown variety and the Krishna tulsi with purplish leaves. *Ocimum sanctum* is susceptible to powdery mildew (*Oidium* sp.), seedling blight (*Rhizoctonia bataticola* (Taub) Butler) (Gupta *et al.*, 1942, Rakshit, 1939–40, Mahmud, 1950–51, Desmukh and Mahmud, 1950–51), and root-knot disease caused by *Meloidogyne incognita*. It is an essential oil bearing plant and commands a superior position in pharmaceutical industries because of its antihelmentic, alexipharmic and antipyretic properties (Haseeb *et al.*, 1999).

The oil is reported to possess antibacterial and insecticidal properties. It inhibits *in vitro* growth of *Myobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*. Towards antitubercular activity, it has about one-tenth potency of streptomycin and one fourth of isoniazid. Rajendhran *et al.* (1998) detected the antibacterial activity of *O. sanctum* against

Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. Sinha and Verma (2002) tested antifungal activities of different plant extracts including *O. sanctum* against *Colletotrichum capsici* and reported encouraging results. The oil obtained from the green variety is active against *Salmonella typhosa*, and the alcohol extracts of leaves are active against *Escherichia coli* (Gupta and Viswanathan, 1955, Gupta *et al.*, 1942, Chopra *et al.*, 1941, Joshi and Magar, 1952, George *et al.*, 1947). Ethanol extract of *O. sanctum* has shown fungitoxic properties against five pathogenic fungi (*Alternaria brassicola*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Shivpuri *et al.*, 1997).

The plant *O. sanctum* is used as a pot herb and its leaves are consumed as a condiment in salads and other food items. Besides the volatile oil, the plant is reported to contain some alkaloids like glycosides, saponins and tannins. The leaves contain ascorbic acid and carotene (Basu *et al.*, 1947, Uphof, 1954). The leaf extract possesses diaphoretic, antiperiodic, stimulating and expectorant properties. It is used in bronchitis; applied to the skin to cure ringworm and other cutaneous diseases; and is dropped into the ear to relieve earache. An infusion of the leaves is used as stomachic in gastric disorders of children. A decoction of the root is given as a diaphoretic in malarial fevers. The seeds are mucilagenous and demulcent, and are given in disorders of genito-urinary system. They contain antistaphylocoagulase which can be extracted with water and alcohol (Kirt and Basu, III 1966-67; Bhat and Broker, 1954). The fixed oil of *O. sanctum* has been found to possess

significant antiulcer activity against aspirin, indomethacin-, alcohol-, histamine-, reserpine-, serotonin- and stress induced ulceration in experimental animal models (Singh and Majumdar 1999). Chauhan (2002) has recently found anti HIV activities of *Ocimum* and other plants which are immunostimulant and can be used in the treatment of AIDS.

The soil, in addition to supporting plants, harbours a large number of plant pathogenic organisms such as bacteria, fungi, insects and nematodes. Nematodes constitute, a largest and ubiquitous group of invertebrates highly diversified with representatives in almost every kind of environment. They occur in unimaginable numbers and show great variation in size and structures. A large number of nematode species are parasites of different kinds of plants and animals. The literature on host-parasite relationship between nematodes and medicinal plants, in general, and between root-knot nematode and *O. sanctum*, in particular, is scanty. Das and Das (1986) reported lesion nematode *Pratylenchus coffeae* from *O. sanctum*.

Root-knot nematodes, *Meloidogyne* spp. are major pests of many important cash crops, including medicinal and aromatic plants (Sasser, 1979; Haseeb, 1994). The root-knot nematodes *Meloidogyne incognita* (Kofoid and White) Chitwood and *Meloidogyne javanica* (Treub) Chitwood have been reported to cause severe losses to different species of *Ocimum* (Haseeb *et al.*, 1993, 1996; Haseeb, 1994). The association of *M. incognita* with *Ocimum* along with the other plants has also been mentioned by Sheela *et al.*, (1996).

The earliest host response in root-knot nematode infection is the formation of discrete galls on the roots of host plant. Molliard (1900) observed galls on the roots of melon, *Coleus* and *Begonia* and reported that after invasion, the root tip growth may be arrested and lateral roots frequently developed near the site of invasion.

Davis and Jenkins (1960) reported gall formation in *Gardenia* spp. infected with *M. incognita* and *M. hapla* in which cortical and stelar proliferation accompanied all infections. Schuster and Sullivan (1960) found galls in tomato caused by *M. incognita* larvae even when they did not enter the roots. The stylet penetrated the root surface cells and secreted materials that stimulated host tissue to form galls. *M. javanica* infection on soybean roots caused hypertrophy, hyperplasia and giant cell formation in the tissue surrounding the head that consequently led to gall formation (Ibrahim and Massoud, 1974). According to Siddiqui and Taylor (1970) gall formation is attributed to hypertrophy of the cortical cells, xylem parenchyma, formation of giant cells, nematode development and egg mass production.

The pathological effects of nematode feeding on crop plants range from simple mechanical injury caused by migration of nematodes between or through plant cells, to complex host-parasite interactions. These plant and nematode interactions cause morphological and physiological changes of the affected tissues in plants. The subsequent development of disease syndrome depends on biochemical reactions in between exudates of pathogens and metabolites, already existing or

produced by the host as a response to infection. The host parasite interaction is a complex developmental system, the failure of compatible response between the two as a result of the activities of metabolic inhibitors may cause death of feeding site and ultimately of the pathogen.

Root-knot nematodes (*Meloidogyne* spp.) attack the underground parts of the plants, where they induce the development of abnormal growth of the stem and the root. Sometimes large galls are developed at the base of the stem. The size and characters of galls vary in different plants as for instance in *Thunbergia laurifolia* and rhubarb, enormously large structures of nearly two feet in diameter were seen (Steiner *et al.*, 1934).

The plant and nematode interactions cause morphological, anatomical and physiological changes of the affected tissues; or death of the cell by removal of their contents; or the host cells adapt to nematode by enlarging or increasing their metabolic activities; or the cells undergo growth or multiplication. These effects of plants have been termed as destructive, adaptive and neoplastic, respectively (Dropkin, 1980).

The root-knot nematodes are sedentary endoparasites of underground parts of the host plants. The sedentary life-cycle is associated with sexual dimorphism (Triantaphyllou, 1966; Davide and Triantaphyllou, 1967; Cohn and Spiegel, 1991). The second-stage juveniles commonly known as larvae, represent the infective stage of *Meloidogyne*. These usually penetrate the

roots and establish their feeding sites in vascular parenchyma. In response to feeding activity, some parenchyma cells become hypertrophied and multinucleate, and are generally known as "giant cells". The term giant cell refers to a multinucleate transfer cell usually induced by root-knot nematode, in which the multinucleate condition results from repeated endomitoses (Endo, 1987). Youssef and El-Nagdi (2004) revealed that second-stage nematode larvae penetrated the roots of fababean by a puncturing action of the stylet. They migrated inter- and intracellularly into the cortex and endodermis damaging the cells in their way. Hypertrophy leads to the formation of giant-cells in cortical and stelar regions. Nemec (1910) noted that the cells in plerome, close to the nematode head, immediately commenced to enlarge their size and increased protoplasmic contents. Their nuclei divided without the division of cytoplasm. Kostoff and Kendall (1930) believed that giant cells were formed by wall dissolution of affected cells followed by coalescence of cell contents. This concept was supported by Christie, 1936; Krusberg and Nielsen, 1958; Dropkin and Nelson, 1960; Littrell, 1966).

Huang and Maggenti (1969a) in their detailed study on giant cell development in *Vicia faba* roots found no evidence of cell wall dissolution or breakdown. They noted that multinucleate condition of giant cell arose from repeated mitosis without cytokinesis of a single diploid cell. Jones and Payne (1978) supported the view of hypertrophy and repeated endomitosis without cytokinesis, while studying early stages of giant cell formation in balsam roots infected with *Meloidogyne*

incognita. They did not find cell wall breakdown or dissolution in the stimulated cells, and concluded that the giant cell originated from a single cell. The intrusions, reported along the giant cell wall, were not the wall fragments but were infoldings of walls of giant cells.

The number of nuclei within the developing giant cell could be correlated with the number of host cells that would normally occupy the volume of the giant cell (Owens and Specht, 1964). Nuclear changes in giant cells range from a nucleus having a hypertrophied nucleolus to nuclei with various stages of membrane deterioration and a lobulated periphery. Other nuclear aberrations included nucleolar fragmentation into fine granules that remained scattered throughout the nucleus. The nuclei having irregular shapes like lobed, dumbbell or sickle shaped have been reported by several workers (Krusberg and Nielsen 1958; Owens and Specht, 1964). Nuclear enlargement was supposed to be due to swelling or due to nuclear fusion. In giant cells of tomato, the nuclear volume increased to six times as compared to the normal $6\mu\text{m}$ (Rubinstein and Owens, 1964). The number of chromosomes in giant cells of tomato inoculated with *M. incognita* was over 100 whereas in normal cells the $2n$ number was 14 (Dropkin, 1965).

The cytoplasm in a young giant cell becomes dense because of abundance of golgi apparatus, mitochondria, ribosomes, polysomes and endoplasmic reticulum. The central vacuole gradually disappears and smaller vacuoles increasingly prevail (Jones and Northcote, 1972; Jones and Dropkin, 1976; Jones and Gunning, 1976; Jones and Payne, 1978; Wergin and

Orion, 1981). Towards the maturity of the nematode its nutrient demand increases and in response the giant cell cytoplasm shows intense metabolic activity. The nuclei become highly lobed and heterochromatic with prominent and numerous nuclear pores, indicating rapid nucleo-cytoplasmic exchange. The starch grains from the giant cells are lost and the secondary vacuoles become more numerous and smaller. Finally, the cytoplasm is extracted as the giant cells senesce, leaving some organelles and the ingrowths, but little ground cytoplasm. Sharma and Tiabi (1989) and Datta *et al.* (1991) reported the giant cell formation within 72 hours of inoculation in the xylem and phloem parenchyma in *Vigna radiata* and *Cyamopsis tetragonaloba*, respectively.

Xylem and phloem tissues become disrupted due to the host parasite relationship of the root-knot nematode infection which causes hindrance in the transportation of water, mineral nutrients and translocation of food materials in the host plants. Formation of giant cells, proliferation of parenchyma cells and multiplication of pericycle and the cells around the nematode head cause the conducting xylem to scatter from its normal path. The vessel elements due to hypertrophic reactions become irregular in shape and size. Small and large parenchyma cells are transformed into vessel like elements and constitute the abnormal xylem. Production of enormous amount of abnormal xylem elements is thought to carry water and nutrients in greater amounts to compensate the loss caused by root-knot nematode infection (Pasha *et al.*, 1987).

The common responses of nematode infestation are similar to those reported for many other plant diseases caused by various pathogens. Enhanced respiration, reduced rate of photosynthesis, stimulated protein and nucleic acid synthesis, accumulation of metabolites at the site of infestation, enhanced enzyme activity and hyperauxinity are some of the physiological responses in the plant that occur due to nematode infestation. The biochemical responses of diseased plants caused by *Meloidogyne* spp. have been investigated by a number of workers. Owens and Specht (1964) and Owens and Bottino (1966), demonstrated that the developing giant cell was the region of intense DNA and RNA synthesis. The galled roots exhibited an unusual increase in the amount of metabolites. Hence, it is evident that root-knot nematodes greatly modify the mode of utilization of minerals by plants for the synthesis of proteins and carbohydrates (Dasgupta and Deb, 1972).

Meloidogyne incognita infection drastically alters the physiology of the host plant leading to the development of characteristic symptoms which are reflected on the shoots and roots. The root infection by *Meloidogyne* spp. causes the formation of feeding site, which acts as a metabolic sink. The food material synthesised in the shoots is mobilized towards the metabolic sink.

Pollution, a serious problem in the present world is defined as an undesirable change in physical, chemical or biological characteristics of our air, land and water that may or will harmfully affect human life or that of desirable species, our industrial processes, living conditions and cultural assets; or

that may or will waste or deteriorate our raw material resources (Odum, 1996). The release of different kinds of pollutants in the air causes air pollution.

Broadly, the air pollutants are categorized into two types: gaseous and particulate. The major gaseous air pollutants are sulphur dioxide (SO₂), nitrogen oxides (NO_x), carbonmonoxide (CO), ammonia (NH₃), chlorine (Cl₂), ethylene (C₂H₄), hydrogen fluoride (HF), ozone (O₃) and peroxyacetyl nitrate (PAN). Particulate air pollutants include coal dusts, fly ash, cement dust and soil dust particles.

Today, India is one among the top ten countries of the world having a very good industrial infrastructure. There are a number of thermal and super thermal power stations in the country which add a lot of pollutants into the air. The air pollution by particulate air pollutants is mainly attributed to both industrial and power generation activities. Fly ash, one of the main particulate air pollutant is emanated from thermal power plants where coal is used as fuel. It is a major cause of ambient pollution, and such a pollution is of great concern in the developing countries (Das, 1986).

Fly ash has shown great potential in enhancing productivity through soil amendments where it acts as a source of trace elements which are beneficial to the plants (Hammermeister *et al.*, 1998). The addition of fly ash to the soil can improve the nutrient status of soil and neutralize soil acidity to a level suitable for agriculture depending upon the initial pH of the soil (Moliner and Street, 1982). There are

various workers who have reported about the promising effects of fly ash on different plants. Mishra and Shukla (1986) reported an increase in plant height, dry weight, metabolic rate and photosynthetic pigments of maize (*Zea mays*) and soybean (*Glycine max*) by dusting fly ash at low rate. Enhancement in seed germination and growth of *Brassica parachinensis* at low levels of fly ash (3 to 6 %) has been reported by Wong and Wong (1989). Wong and Su (1997) found improved seedling emergence and dry weight of *Agropyron elongatum* due to addition of fly ash in soil. The growth promising effects of fly ash amendment in soil have been reported in tomato and *Phaseolus aureus* (Khan and Khan, 1996; Kumar *et al.*, 1998, respectively). High fly ash concentration suppresses plant growth and causes deterioration of soil properties (Hodgson and Holliday, 1966; Adriano *et al.*, 1980).

Among other potential factors air pollution is important for growth and reproduction of microorganisms. The air pollutants have direct and indirect effects on microorganisms. The plant diseases, caused by microorganisms, are either aggravated or suppressed, depending upon the nature of disease, the type of the host and the concentration and diversity of pollutants. Singh (1993) observed that higher concentration of fly ash suppressed the growth and development of root-nodule bacteria (*Bradyrhizobium japonicum*) and root-knot nematode (*Meloidogyne javanica*). Decrease in soil population of *M. javanica* at 10 to 100 % fly ash has been reported by Pasha *et al.*, (1990). Certain elements such as potassium, phosphorous and boron play important roles in defence mechanisms of

plants against nematodes (Kirkpatrick *et al.*, 1964; Francois, 1984). All these elements are amply present in fly ash (Elseewi *et al.*, 1981; Druzina *et al.*, 1983; Wong and Wong, 1989).

The continuous improvement is one of the underlying promise of agricultural as well as plant sciences. With the rapid development of knowledge and technology in the fields of plant pathology over the last few years, new opportunities for addressing crop improvement are now feasible. Since the root-knot nematode, *M. incognita* is a devastating pathogen, that drastically alters the physiology of the plants after infection, therefore, it needs to be managed. The fly ash on the other hand increases the nutrient status of soil and under certain circumstances can be used as a fertilizer. It also releases certain useful elements which keep a check on the disease development. Keeping these aspects under consideration, it was felt desirable to check out the pest status of the root-knot nematode on *O. sanctum*. For this purpose, following experiments were performed to compare and confirm earlier reports and to provide some new information.

SECTION I

Experiment 1: Histopathological response of *Ocimum sanctum* to *Meloidogyne incognita*.

SECTION II

Experiment 2: Effect of different inoculum levels of *Meloidogyne incognita* on growth and yield of *Ocimum sanctum*, reproduction

of the nematode and internal structure of root.

Experiment 3: Effect of different inoculum levels of *Meloidogyne incognita* on growth, chlorophyll and oil contents of *Ocimum sanctum*.

Section III

Experiment 4: Effect of fly ash amended soil on the plant growth, yield, chlorophyll pigment and oil content of *Ocimum sanctum*.

Experiment 5: Effect of fly ash amended soil on the development of the root-knot nematode (*Meloidogyne incognita*) and growth of *Ocimum sanctum*.