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BIODIVERSITY OF SOIL NEMATODES AND THEIR BENEFICIAL ROLES IN AGRICULTURE

Zakaullah Khan*

Abstract

Nematodes are roundworms in the Phylum Nematoda. Soil is an excellent habitat for nematodes, and 100 cc of soil may contain thousand of them. Most of soil nematodes do not parasitize plants, they play important and beneficial role in decomposition of soil organic matter, nutrient recycling and disease control. Soil inhabiting nematodes can be categorized according to their feeding habits, which will be useful to ecologists in understanding the role of nematodes in soil food webs. Plant parasites belonging to orders Tylenchida, Aphelenchida and Dorylaimida, possess spear-like feeding organ, stylet to puncture cells for feeding. Many free-living nematodes which are abundant in soil are bacterivores, feed only on bacteria, possess a hollow feeding tube for ingestion of bacteria. The bacterivores are beneficial in the decomposition of organic matter. Fungivores have weak hollow stylet for piercing the fungal hyphae and sucking the contents, they belong to the orders Tylenchida, Dorylaimida, and Aphelenchida. The feeding habits of these nematodes at times protect plants from pathogenic fungi. Fungivores and Bacterivores play important in organic decomposition in soil, and contribute to maintaining adequate levels of plant-available nitrogen in farming systems. Predatory nematodes feed on other soil nematodes including plant parasitic. Some of the nematodes are considered as omnivores which devour on fungal spores as well as bacteria and dead organic matter.

Key words : - Nematodes, Parasities, Predators, Omnivores. Biological control

Introduction

Nematodes are microscopic, wormlike organisms and are one of the most abundant metazoans in soil. They are next only to arthropods in species diversity and their population densities in soil may reach 10 millions of individuals per m^2 (Lavelle and Spain, 2001). Most research on soil nematodes has focused on the plant-parasitic nematodes that attack the roots of cultivated crops. Less attention has been given to nematodes that are not plant-feeders and play beneficial roles in the soil environment. Nematodes can be classified into functional groups based on their feeding habits, which can often be deduced from the morphology of their mouthparts (Fig.1).

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In agricultural soils, the most common groups of nematodes are the bacterial-feeders, fungal-feeders, plant parasites, predators, and omnivores (Yeates *et al.*, 1993). All these types of nematodes coexist in soil and contrary to their notorious image as hidden enemies of farmers; some nematode trophic groups play an important role in organic matter decomposition, mineral and nutrient cycling, and control of pests and diseases. They are excellent indicators of pollution, and metazoan models for basic studies in developmental biology, neurobiology, genetics, and aging, and for understanding the effects of nutrients on reproduction, development, and growth.

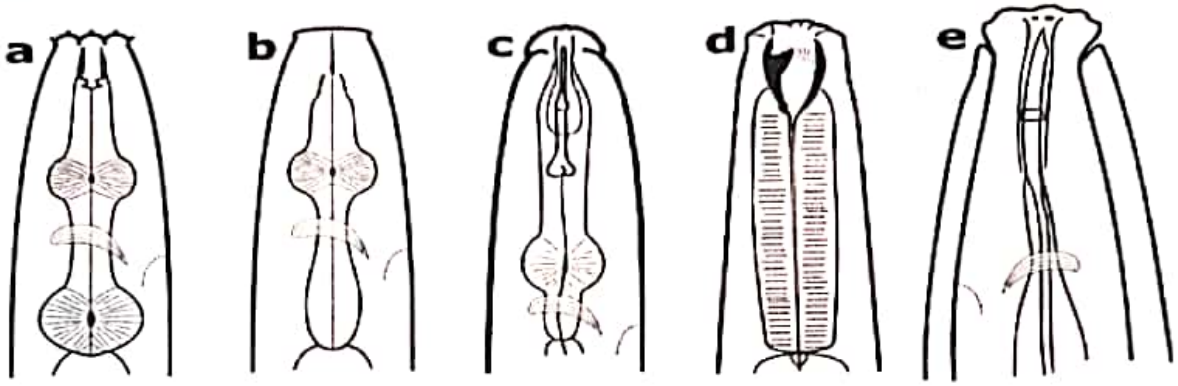

Fig. 1. Nematodes can be classified into different feeding groups based on the structure of their mouthparts. (a) bacterial feeder, (b) fungal feeder, (c) plant feeder, (d) predator, (e) omnivore.

Role of Nematodes in Insects and Slug Control

Many species of nematodes are pathogenic to invertebrates and species from more than 30 families are pathogenic to insects. Nematode species in families Steinernematidae, Aphelenchidae, Allantonematidae, Neotylenchidae, Rhabditidae, Heterorhabditidae, Mononchidae, Mermithidae, Parasitylenchidae, Iotonchidae, Tetradonematidae, Sphaerulariida, Phaenopsitylenchidae, Dorylaimidae and Nygolaimidae, are potential biocontrol agents of insect pests (Poinar 1979; Kaya 1993).

Tetradonematids are parasites of many species of insects in the orders Diptera, Hymenoptera, and Coleoptera. Some species are potential biocontrol agents of insect pests. For example, *Tetradonerna plicans* is an endoparasite of sciarid flies. It has high pathogenicity, reproductive potential and good shelf-life. It is also easy to produce *in vivo*. Beddingia. (syn. Deladenus) siricidicola has been successfully used for the control of Siricid wasp (Sirex noctilo) in Australia and New Zealand (Bedding 1992). This nematode species has a parasitic heterosexual and a free living myceliophagus life cycle. It is easy to culture and has high infectivity. However, many nematode species in the families Allantonematidae, Parasitylenchidae, Iotonchidae, Tetradonematidae, Sphaerulariidae, and Phaenopsitylenchidae have not been tried for biological control of insects primarily because of their complex life cycles and difficulties in culturing the nematodes. In India, species of *Paraiotonchium*,

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Mononchids: The mononchid possess a strongly sclerotized buccal cavity, which is often armed with one or more large puncturing tooth or numerous small grasping teeth/denticles or both. Several of commonly occurring mononchids feed extensively, though not exclusively, on plant parasitic and other nematodes. They may swallow their prey whole if it is of smaller size, or at times feed by cutting larger prey into pieces. The duration of the life cycle varies from species to species. To complete one generation, *Prionchulus punctatus* takes 45 days while *Mononchus aquaticus* takes only 15 days at 25ºC (Maertens, 1975). Small (1979) reported significant reductions in the population densities of potato cyst nematode, *Globodera rostochiensis* and root-knot nematode, *Meloidogyne incognita* in the presence of a predatory nematode, *P. punctatus*, in pot experiments. Rama and Dasgupta (1998) reported that true predator-prey relationship existed between *Tylenchulus semipenetrans* and *Helicotylenchus dihystera* with *Iotonchus temicaudatus* in mandarin orange orchards. These reports indicated that mononchids are already providing natural control of plant parasitic nematodes in soil. If their population can be manipulated in the field then they can be used as successful biocontrol candidates. The main drawback in using mononchids as biological control agent lies in their poor adaptability to environmental fluctuations such as, temperature, moisture, soil pH and texture. Besides, their low rate of reproduction and longer life cycle and cannibalistic behaviour are other hindering factors.

Dorylaimids: Dorylaimids possess a hollow stylet, properly called as odontostyle with which they puncture the prey organisms on which they feed and through which they suck food. Linford and Oliveira concluded that, when feeding on other nematodes, the large odontostyle of these predators disorganizes the internal organs of the prey as to quickly render it immobile. Generally dorylaimids take 3-6 months to complete one life cycle, while *Labronema vulvapapillatum* completes life cycle in 36 days at 25ºC (Ferris, 1968;). The detection of *Eudorylaimus obtusicaudatus* feeding on eggs inside cysts of *Heterodera schachtii* and an increase in the population of *Thornia* sp. in the presence of citrus nematodes but a decrease in their absence in pot trials (Boosalis and Mankau, 1965) have indicated their biocontrol potential against plant parasitic nematodes. The most advantageous and encouraging aspect of dorylaimids is that it is easy to maintain their populations simply by adding organic matter to agricultural fields; as they are polyphagous in nature, they will remain abundant in soil without prey nematodes.

Diplogasterids: The diplogasterid predators posses comparatively a smaller buccal cavity than those of mononchids but armed with a strongly built, movable, dorsal tooth. They feed on nematodes, bacteria and other soil microorganisms. Diplogasterids are generally found abundantly in decomposing organic manure. They complete their life cycles in 8-15 days and are the most readily cultured nematodes, being easily maintained on simple nutrient media containing bacteria (Yeates, and wardly 1996). Studies on predatory behavior of diplogasterid predators on plant parasitic and other soil dwelling nematodes revealed their strong chemotaxis behaviour towards attractants from prey nematodes (Steel *et al.*, 2011). Fauzia *et al.* (1998) demonstrated the ability of *M. longicaudatus* to reduce root galling by root-knot nematodes in pot tests, resulting in improved vegetative growth and increased root mass. Further Khan and

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Kim (2005) reported that a pre-planting application of *M. fortidens* in potted field soil infested with root-knot nematode, *M. arenaria* reduced the root galling on tomato plants and suppressed the *M. arenaria* population. Bilgrami *et al.* (2008) have demonstrated that *M. gaugleri* reduced the population of naturally occurring plant parasitic nematodes in a turf grass field in USA.

The hyphal feeder nematodes' belong to the orders Tylenchida, Dorylaimida, and Aphelenchida. They have weak, hollow stylet for piercing the fungal hyphae and sucking the contents. The feeding habits of these nematodes sometimes protect plants from pathogenic fungi. For example, Aphelenchoides hamatus and A. hylurgi feed on plant pathogenic fungi (Zunke et al. 1986; Bird et al. 1989); and damping off disease of cucumber (Cucumis sativus) by Rhizoctonia solani is less severe in the presence of *Aphelenchus avenae*. In nature, the hyphal feeder nematode may be responsible for significant natural control of fungal pathogens. Like wise, bacterial feeder nematodes may reduce soil borne bacterial diseases by lowering the primary bacterial inoculum. Addition of organic matter to agricultural soils increases the populations of hyphal and bacterial feeder nematodes (Ishibashi and Kondo 1986).

Role of Nematodes in Organic Matter Decomposition

Decomposition Saprophytic nematodes comprise 52% of the total nematode genera (Wharton 1986). They are less studied than the parasitic species. The quality and quantity of organic matter affect distribution and abundance of these nematodes. The organic Matter provides an energy source for growth of bacteria and the bacteriophagus nematodes feed on-the bacteria. The soil nematodes, especially bacterial- and fungal-feeding nematodes, can contribute to maintaining adequate levels of plant-available N in farming systems relying on organic sources of fertility (Ferris et al., 1998). The chemotropic bacteria convert the excretory product of nematodes (i.e., ammonia) to nitrate and nitrite compounds. The predominance of bacteriophagus nematodes in agricultural systems indicates faster rate of mineralization, decomposition, and nutrient turn over (Freckman 1988). Nematodes contribute directly to nutrient mineralization through their feeding interactions. Both bacterivore and fungivore nematodes mineralize N in soil (Ferris et al., 1998; Chen and Ferris, 1999), bacterial-feeding nematodes consume N in the form of proteins and other N-containing compounds in bacterial tissues and release excess N in the form of ammonium, which is readily available for plant use. Indirectly, nematodes enhance decomposition and nutrient cycling by grazing and rejuvenating old, inactive bacterial and fungal colonies, and by spreading bacteria and fungi to newly available organic residues. Soil mineral N levels are increased by 20% or more by the feeding of bacterial- and fungal-feeding nematodes in microcosm experiments (Ferris et al., 1998; Chen and Ferris, 1999). Under the dry fallow conditions, bacterivore and fungivore nematodes decline in abundance due to lack of soil moisture and food (Ferris et al., 2004). It may be conclude that conditions conducive for activity of the soil food web can be created through irrigation in the late summer. Growing a cover crop during the late summer irrigated period will generally further enhance activity in the soil food web. Sun hemp consistently increased the abundances of bacterivorous and fungivorous nematodes at cover crop incorporation (Marahatta et al., 2010). The bacteriophagus nematodes have an unarmed open stoma. The

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bacteriophagus nematodes have a short life span and generation time varies from few days to few weeks depending on the species; for example, *Mesodiplogaster lheritieri* completes its life cycle in 4 days and *Acrobeloides* sp in 11 days (Anderson et al. 1981). They are more abundant in undisturbed soils than in heavily-managed agricultural systems (Yeates 1996).

Role of Nematodes in Nutrient Cycling Carbon Cycle

The decomposition of plant material can be studied by measuring weight loss, directly or by monitoring the "CO2 produced from "C labeled material (Lynch 1983). The total nematode community (0.3×10^6 to 9.0×10^6 nematodes m^{-2}) respires comparatively lesser of the total carbon input in natural ecosystems (0.6-0.9%) than in agroecosystems (1.3-2.0%) (Sohlenius *et al.* 1988). The bacteriophagus nematodes indirectly stimulate the rate of organic matter decomposition by grazing (Freckman et al. 1987). In a microcosm study on *Pseudomonas* sp and*Mesodiplogaster* sp, the microcosms with nematodes and bacteria had greater CO_2 output than those with bacteria alone (Anderson *et al.* 1981). Similarly, free-living marine nematodes (e.g., *Diplolaimelloides* spp) may increase the rates of carbon mineralization by 300% (Findlay and Tenore 1982).

Nitrogen Cycle: It is estimated that 60% of nitrogen ingested by the total nematode community comes from bacteria, 15% from fungi, and 25% from plant roots. The bacteriophagus nematodes and amoeba together account for over 83% of nitrogen mineralization (Hunt et al. 1984; Griffiths 1990). The nematodes have been related to increased plant production owing to increased nitrogen availability (Ingham et al. 1985).

Sulfur Cycle: There are very few studies on the role of nematodes in sulfur cycling. The primary effect of nematodes on sulfur cycling and biogeochemical cycling is through interactions with sulfur-oxidizing bacteria. An efficient anaerobic trophic food web exists near natural oil and petroleum sources. The hydrogen sulfide produced is oxidized by*Beggiatoa* species of sulfur-reducing bacteria. The bacteriophagus nematodes feed on these bacteria and are the key intermediaries between the bacterial and macrofaunal predation .

Phosphorus Cycle: Increased mineralization of carbon, nitrogen, and phosphorus owing to grazing by free-living nematodes has been studied but the exact mechanisms are not yet known.

Nematodes as Biological Indicators: Biomonitoring is an application of one or more biological systems as indicators of the quality of contaminated environments. Nematodes are useful as biological indicators because of the following characteristics.

- Nematodes are metazoans with well defined organ systems as opposed to bacteria and protozoa.
- They are small animals with short generation duration, allowing them to respond quickly to changes in food supply.
- They are relatively easy to culture.

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- They are ubiquitous; even in polluted or disturbed areas they are usually the last animals to die.
- They appear to be relatively stable populations; thus any change can be viewed as environmental disturbance.
- They respond to disturbances in habitat by changing trophic structure.
- They are easy to sample than other microfauna and macrofauna.
- Low cost is associated with sampling and identification of nematodes.
- They survive desiccation and revive with suitable moisture.

Some methods have been developed to assess the pollution levels using nematode assays. Two of such assays are described.

The *Panagrellus* assay the bioassays using the nematode *Panagrellus redivivus* have wide applicability in a biomonitoring system particularly in rapid assessment of aquatic contaminants. The assay is relatively simple. Sterile second stage juveniles are grown on a defined media with and without added toxicant. After 96 h the survivors are counted and lengths of each developmental stage are measured. Genetic assays of mutagenesis are determined using mutant strain of *Panagrellus*. The period of development requires extensive gene expression and the growth of nematode is highly sensitive to known mutagens. The Panagrellus bioassay can discriminate different types of toxic effects: lethality - where a significant proportion of test population dies, relative to the number that dies in the control population; inhibitory - where a significant proportion of the test population fails to grow to the fourth stage juvenile or adult stage relative to the control population; stimulatory - where significantly more of the test population reaches the fourth stage juvenile and adult stage than in control population; and phenotoxic - where the tested material, specifically inhibits the completion of the fourth stage juvenile or adult molt. Each of this effects can be quantified and the combined effects can be expressed as a single value-fitness (a weighted mean of the survival, growth, and maturation of the test population) (Samoiloff and Bogaert 1984).

The nematode-copepod ratio Nematodes and copepods respond very quickly to pollution but differ in their sensitivity to pollution. The nematodes are less sensitive than copepods; so the high nematode:copepod ratioindicates pollution. The potential of nematode:copepod ratio as pollution indicator has been observed in sewage system and in various organic pollutants (Raffaelli and Mason 1981).

Nematodes as Models

Cell biologists believe that the basic life processes proceed along similar paths in animals. This idea has given impetus to use of nematodes as models for studying aging in animals. In some important respects, age-related changes in nematodes have been shown to closely parallel that in mammals (Zuckerman 1987). Nematodes have constant number of cells with cell division occurring only in reproductive and intestinal cells. Effects of nutritional

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supplements can be directly related to effects on post-embryonic and adult development or aging and it is not influenced by changes due to cell turn over. The free-living nematodes *Caenorhabditis* sp, *Turbatrix* sp, and *Panagrellus* sp have been exclusively used for these studies. *Caenorhabditis elegans* is widely used in developmental biology, neurobiology, and genetics. The advantages of using these nematodes include availability of simple axenic and monoxenic culturing techniques, a choice of several methods for obtaining synchronous groups of nematodes from cultures of mixed age group, short life span, differentiated organ systems, and small size (Zuckerman 1987).

Conclusion

The beneficial role of nematodes in agro-ecosystems has not received much attention as of plant parasitic group. But the presence of many groups of beneficial nematodes in to soil is vitally important in soil ecosystem process. The insect parasitic nematodes have been used for controlling the insect pests in industrialized countries but not in the developing countries. Predatory nematodes used to manage plant parasitic nematodes especially in greenhouses and pot cultures. The contributions of nematodes to organic matter decomposition and nutrient recycling, and their use as indicators of soil health deserve much greater attention of scientific community world wide.

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PREPARATION OF 4F COMPLEX WITH BIS BENZIMIDAZOLE LIGANDS : SPECTROSCOPIC AND BIOLOGICAL STUDIES

Armeen Siddique* ,Prof. Zafar A Siddiqi**

Abstract

The novel mixed ligands complexes were characterized employing analytical and spectral IR, ^{1}H and ^{13}C NMR and EPR techniques. In order to exploit them as possible antimicrobial and luminescent agents, the fluorescence and antimicrobial studies have also been performed.

Key words : - Mixed Ligands, Intra Red Spectroscopy an microbial

Introduction

The coordination compounds derived from bio-mimic ligands have attained considerable attention by inorganic and bio-inorganic chemists. A number of such complexes have been exploited to serve as models for metallo-proteins and enzymes. Ligands having resemblance to the macro-biomolecules have been prepared and characterized in literature (Chen et. al.1994) by bio-coordination chemists in this regard. Imidazoles/ benzimidazole derivatives have shown a broad biological significance (Quiroz-Castro et. al. 2000) and this moiety acts as a potential coordinating/ chelating agent towards the metal ions. The coordination ability of a number of monodentate benzimidazole derivatives has been thoroughly investigated and reported (Goodgame et. al. 1992) in literature.

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Metal complexes of polyfunctional benzimidazole and its derivatives have attracted the attention as they are capable to generate supramolecular self-assembly via inter-ligand hydrogen bonds Sun et. al. (2002). The nature of linking group between the benzimidazole moieties in such ligands as well as that of the counter anions in the complexes probably plays important role in the supramolecular architecture Raj et.al.(2001) & Akutagava et. al. (2003).

Preparation of Ligand (L¹), [1,2-Bis(benzimidazole-2-yl)ethane dihydrochloride]

To O-phenylenediamine (10.81 g, 100mmol) and succinic acid (5.90 g, 50 mmol) was added 4 M hydrochloric acid (120 cm^3). The mixture was refluxed for 17 h at 120 °C and gradually cooled to room temperature, at which point the hydrochloride separated as crystalline solid. The solid was filtered, washed with acetone and dried under vacuum at 60°C. Yield (11.29 g, 69%). Anal. found: C, 57.2; H, 4.8; N, 16.7%. IR (KBr): 3426m (br), 3019-2612s (br), 1623s, 1573s, 1511s, 1481w, 1461s, 1435m, 1380s, 1299m, 1264m, 1229s, 1184w, 1107w, 1020w, 924m, 898m, 817s, 763w, 732s, 620s, 510m, 433m cm^{-1}.

General procedure for the synthesis of the complexes

An ethanolic solution (10 mL) of $LnCl_3 \cdot 6H_2O$ was dropped to a magnetically stirred hot solution of the ligand **(L)** (0.674g, 2 mmol) taken in 20 mL ethanol. The stirring was continued for 1 h. at room temperature giving precipitates in the solution. The precipitate was filtered off, washed with ethanol and dried under vacuum. Attempts for the recrystallization of the complexes in different solvents could not provide single crystals suitable for X-ray crystallographic studies.

$[La(2L^1)Cl_3].H_2O$ (1)

[White, m.p. >300°C, yield 45%,]. Anal. Calcd. for $C_{32}H_{30}N_8OLaCl_3$: C 48.78, H 3.84, N 14.22%. Found: C 48.75, H 3.80, N 14.19%. Molar conductance, Λ_m (in 10^{-3} M CH_3OH): 29.4 $ohm^{-1}cm^2mol^{-1}$. ESI-Mass: *m/z* = 789 (8%), $[La(2L^1)Cl_3H_2O+2H]^+$; *m/z* = 788 (15%), $[La(2L^1)Cl_3H_2O+H]^+$; *m/z* = 787 (20%), $[La(2L^1)Cl_3H_2O]^+$; *m/z* = 680 (4%), $[La(2L^1)H_2O+H]^+$; *m/z* = 679 (10%), $[La(2L^1)H_2O]^+$; *m/z* = 547 (27%), $[La(L^1)(L^1/2)H_2O-H]^+$; *m/z* = 526 (36%) $[La(L^1)(L^1/2)-3H]^+$, *m/z* = 525 (99%), $[La(L^1)(L^1/2)-4H]^+$; *m/z* = 363(11%), $[La(BImz)_2-2H]^+$.

$[Pr(2L^1)Cl_3].H_2O$ (2)

[White, m.p. >300 °C, Yield 52%]. Anal. Calcd. for $C_{32}H_{30}N_8OPrCl_3$: C 48.66, H 3.83, N 14.19%. Found: C 48.60, H 3.83, N 14.10%. Molar conductance, Λ_m (in 10^{-3} M CH_3OH): 27.5 $ohm^{-1}cm^2mol^{-1}$. ESI-Mass: *m/z* = 789 (4%), $[Pr(2L^1)Cl_3H_2O]^+$; *m/z* = 788 (10%), $[Pr(2L^1)Cl_3H_2O-H]^+$; *m/z* = 787 (17%), $[Pr(2L^1)Cl_3H_2O-2H]^+$; *m/z* = 714 (5%), $[Pr(2L^1)H_2OCl]^+$;

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m/z = 680 (18%), $[Pr(2L^1)H_2O+H]^+$; *m/z* = 679 (21%), $[Pr(2L^1)H_2O+2H]^+$; *m/z* = 548 (22%) $[Pr(L^1)(L^1/2)H_2O]^+$, *m/z* = 547 (51%), $[Pr(L^1)(L^1/2)H_2O-H]^+$; *m/z* = 525 (99%), $[Pr(L^1)(L^1/2)-4H]^+$; *m/z* = 263 (72%), $[Pr(L^1/2)]^+$.

$[Nd(2L^1)Cl_3].H_2O$ (3)

[White, m.p. >300 °C, Yield 53%]. Anal. Calcd. for $C_{32}H_{30}N_8ONdCl_3$: C 48.45, H 3.81, N 14.13%. Found: C 48.41, H 3.10, N 14.11%. Molar conductance, Λ_m (in 10^{-3} M CH_3OH): 25 $ohm^{-1}cm^2mol^{-1}$. ESI-Mass: *m/z* = 789 (4%), $[Nd(2L^1)Cl_3H_2O-3H]^+$; *m/z* = 788 (11%), $[Nd(2L^1)Cl_3H_2O-2H]^+$; *m/z* = 787 (22%), $[Nd(2L^1)Cl_3H_2O-H]^+$; *m/z* = 680 (9%), $[Nd(2L^1)H_2O+2H]^+$; *m/z* = 679 (19%), $[Nd(2L^1)H_2O+2H]^+$; *m/z* = 548 (22%), $[Nd(L^1)(L^1/2)H_2O]^+$, *m/z* = 547 (50%), $[Nd(L^1)(L^1/2)H_2O-H]^+$; *m/z* = 525 (99%), $[Nd(L^1)(L^1/2)-4H]^+$; *m/z* = 404 (5%), $[Nd(L^1)(CH_2)-3H]^+$; *m/z* = 377 (10%), $[Nd(BImz)_2(CH_2)+H]^+$; *m/z* = 263 (68%), $[Nd(BImz)(CH_2)+4H]^+$; *m/z* = 145 (99%), $[Nd(CH_2)]^+$.

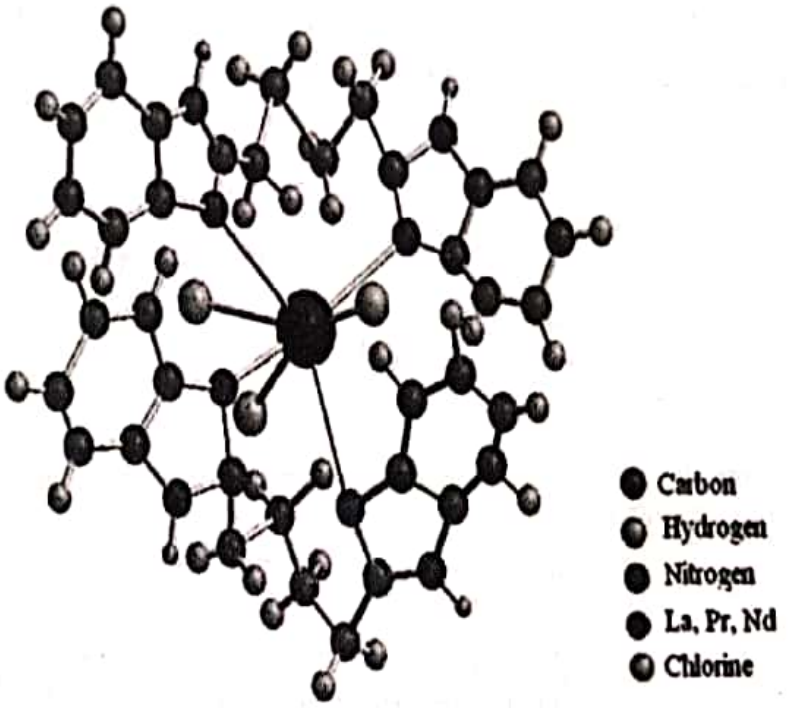

Fig:1 complexes of (1-3)

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Calculations

The nephelauxetic effect is a measure of the degree of covalency (β_{av}) of the M–ligand bond(s) in the complexes. There is a considerable reduction in the magnitude of the radial integral regarding the metal ions valence orbitals in the process of complex formation. Nephelauxetic ratio, β_{av} can be calculated from the relation

$$\beta_{av} = \frac{1}{2}\sum_{n=1}^{n}\frac{\nu_{comp}}{\nu_{aq}} \qquad (1)$$

where υ_{comp} and υ_{aq} are the energies (cm^{-1}) of the f–f bands observed in the complexes and their aquo counter parts, respectively. The f-orbitals when involved in covalent bond formation with the ligand, the metal ion 4f wave function (φ_{4f}) is expressed (Tondon and Mehta, 1970)

by the expression

$$< \phi_{4f}| = (1-b)^{1/2} < 4f| - b^{1/2} < \phi_{ligand}| \qquad (2)$$

where $b^{1/2}$ measures the amount of 4f–ligand orbital mixing and is calculated (Henrie and Choppin ,1968) from the relation

$$b^{1/2} = \left[\frac{(1-\beta_{av})}{2}\right]^{1/2} \qquad (3)$$

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Table 1. Important frequencies (cm^{-1}) in IR spectra of the ligand (L) the complexes (**1–3**) with assignments.

Compound	υ(NH)	υ(C=N)$_{ring}$	υ(C=C)$_{ring}$	υ(M–N)	υ(M–Cl)
(L[1])	3211_s	1600_s	1438_s 761_s		
(1)	3162_{bw}	1627_s	1456_s 761_s	476_m	231_m
(2)	3174_{bm}	1627_s	1456_s 762_m	440_m	245_m
(3)	3213_{bm}	1632_s	1457_s 762_m	464_m	246_w

m = medium, s = sharp, b = broad, w = weak

Antimicrobial studies

The in-vitro antimicrobial activities of the ligand (L) and the complexes (**1–3**) were screened against the fungi Aspergillus niger (AN) and Penicillium notatum (PN) and the bacteria Pseudomonas aeruginosa (PA) and Bacillus cirroflagellosus (BC) using the standard method. Greseofulvin and Norfloxacin were used as standards against fungi and bacteria respectively. 1 mg/mL of the ligand, complexes or the standard in DMSO was employed for the experiments. Separate experiments were performed to verify the activity of the solvent (DMSO) as control. The cultures of the fungi and the bacteria consisted of peptone (0.6%), yeast extract (0.3%), beef extract (0.13%) and nutrient agar. The nutrient agar further consisted of definite volumes of peptone (0.5%), yeast extract (0.15%), beef extract (0.15%), NaCl (0.35%), dipotassium phosphate (0.36%) and potassium dihydrogen phosphate (0.13%). Wells were made by scooping out the nutrient agar with a sterile cork borer. The solutions of the test compounds (0.1 mL) were added to the wells using sterile pipettes. The plates were further incubated at 37 °C for 48 h. The antimicrobial activity was estimated on the basis of size of inhibition zone formed around the wells in the plates. It was observed that the ligand (L) is less active against both the fungi and the bacteria. In case of AN, the complexes (**1–3**) showed comparable activity to that ligand (L). However, in case of PN, enhanced activity compared to the ligands (L) was observed for the complex (**3**) only. The complexes exhibited moderately high activities against the gram +ve bacteria (BC) compared to the ligands (L). However, it was indicated from the experiments that for the gram –ve bacteria (PA), the complexes do not exhibit any activity compared to the ligand. This inactivity stems from the higher lipid content in the cell membrane of PA compared to BC

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which prevents easy diffusion of complex into the cell. On comparison with the ligand the complexes were found to have increased activities (Table 2) which are attributed to the synergistic effect that increases the lipophilicity of the complex. The increased lipophilicities of complexes permit easy penetration into lipid membranes of organisms and facilitates blockage of metal binding sites in enzymes.

Table 2. Antimicrobial screening of the ligands (L) and the complexes (**1–3**)

Compound	Fungi		Bacteria	
	AN	PN	PA(gram –ve)	BC(gram +ve)
Ligand (L^1)	+	+	+	+
$[La(2L^1)Cl_3].H_2O$(**1**)	+	+	-	++
$[Pr(2L^1)Cl_3].H_2O$(**2**)	+	+	-	++
$[Nd(2L^1)Cl_3].H_2O$(**3**)	+	++	-	++
Grisofulvin	+++	+++	-	-
Norfloxacin	-	-	+++	+++
Control	-	-	-	-

Key to interpretation: – = no activity; + = less active; + + = moderately active; + + + = highly active, AN = Aspergillus niger, PN = Penicillium notatum, PA = Pseudomonas aeruginosa, BC = Bacillus cirroflagellosus.

Conclusion

The organic moiety 1,2- Bis(benzimidazole-2-yl)ethane dihydrochloride, used as ligands in the present work is quite reactive towards lanthanide salts forming complexes characterized from spectral studies. The present studies indicate that the complexes attained a hepta coordinate geometry which was further supported from molecular model computations. Some of the complexes exhibited increased activity against selective bacterial and fungal stains compared to the free ligand.

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SEMICLASSICAL MODEL FOR THE ANISOTROPIC THERMAL TRANSPORT IN III-V SEMICONDUCTOR SUPERLATTICES

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Abstract

This paper presents a thermal conductivity of III-V semiconductor superlattices (SLs). An effective interface rms roughness is the only adjustable parameter. The in-plane thermal conductivity is obtained from the layer conductivities connected in parallel. The cross-plane thermal conductivity is calculated from the layer thermal conductivities in series with one another and with thermal boundary resistances (TBRs) associated with each interface; the TBRs dominate cross-plane transport. The model is applied to multiple III-arsenide superlattices, and the results are in very good agreement with experimental findings. The method is simple and accurate, easy to implement, and applicable to complicated SL systems.

Key Words: Semiconductors, arsenide superlattices, thermal conductivities.

Introduction

Theoretical studies find that the diffuse interface scattering is responsible for lowering of the in-plane (and, in part, the cross-plane) thermal conductivity, while the thermal boundary resistance (TBR) between adjacent layers is a key factor in the cross-plane thermal-conductivity reduction (Chen, 1998 & 1997). Superlattices based on III-V compound semiconductors have widespread use in optoelectronics (Faist et. al., 1994). In quantum cascade lasers (QCLs), self-heating is the main issue limiting the development of room-temperature (RT) continuous-wave lasing, which is exacerbated by the poor thermal conduction through hundreds of interfaces in a typical structure (Vitiello et. al. 2008).

A good understanding of the influence of interfaces on the thermal conductivity tensor of III-V SLs would enable advances in the design and modeling and optoelectronic devices for enhanced reliability. The interfacial transport behavior is largely dependent on the material system and interface quality (Cahill et. al. 2014). The acoustic mismatch model (AMM) and the diffuse mismatch model (DMM) have been traditionally used to calculate the phonon transmission coefficient and the resulting TBR of an interface. These two models yield the lower and upper limits of the TBR, respectively, but do not satisfactorily explain realistic experimental results (Cahill et.al. 2003). Molecular dynamics simulations have provided valuable insights into heat transport across a number of solid-solid interfaces.

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The nonequilibrium Green's function technique (NEGF) has also been applied to describe the phonon dynamics, generallywithout phonon-phonon scattering. In general, atomistic simulations are limited by computation burden, which makes it hard to study complicated SL structures, such as the active region of solid-state lasers (Faist et. al., 1994). In this paper, we present a semiclassical model describing the full thermal conductivity tensor of III-V compound SL structures and apply it to III-arsenide systems. The phonon transport inside each layer is captured by solving the phonon Boltzmann transport equation (PBTE) in the relaxation-time approximation (RTA), with rates describing the common internal scattering mechanisms as well as the partially diffuse scattering from the interfaces. The inplane thermal conductivity is obtained from the layers connected in parallel, while the cross-plane conductivity is calculated from the layers and TBRs in series. Nanoscale thermal transport is of considerable importance in the operation of modern electronic, optoelectronic, and thermoelectric devices (Pop, 2010).

THERMAL CONDUCTIVITY OF III-V SUPERLATTICES

A semiconductor SL is a periodic structure, with each period consisting of two or more thin layers of different materials. III-V semiconductor SLs have been widely used in electronic and photonic devices. Experimental results on several material systems show that the thermal conductivity of a SL is substantially lower than that of a weighted average of the constituent bulk materials Panzer et. al. (2009) few transition layers between adjacent materials in a SL

As mentioned briefly above, there will inevitably exist a structure (Chen, 1994). Figure 1 shows a schematic of interfaces between lattice-matched crystalline layers in SLs. In the transition region, if we drew a line that separated the atoms of one crystal from those of the other, we would get a jagged boundary. Therefore, we model the interface with an effective interface rms roughness D, which captures the basic properties of interfacial mixing. The thicker the transition layer, the higher the D. Most III-V SLs are grown by molecular beam epitaxy (MBE) Cheng (1997) or metal-organic chemical vapor deposition (MOCVD), Goetz et. al. (1983) both well-controlled growth.

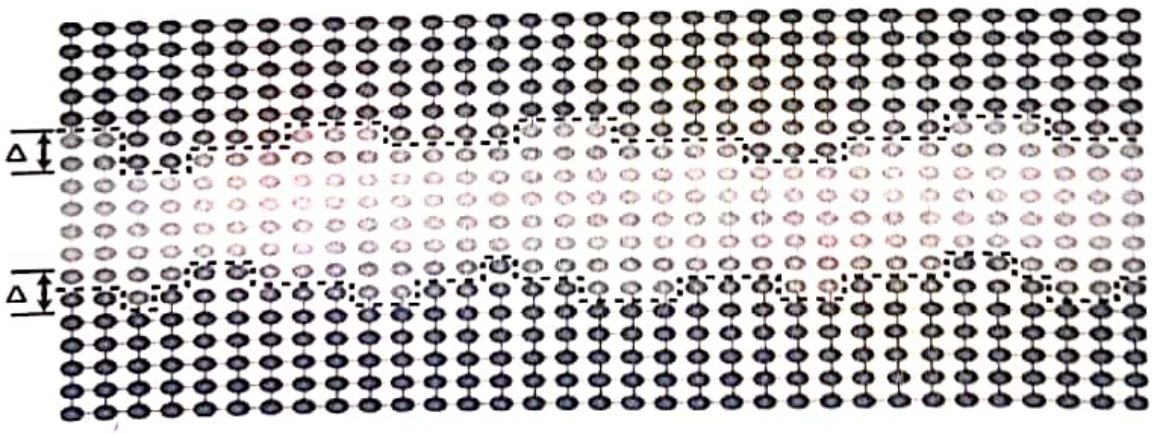

FIG. 1. Even between lattice-matched crystalline materials, there exist nonuniform transition layers that behave as an effective atomic-scale interface roughness with some rms height D. This effective interface roughness leads to phonon-momentum randomization and to interface resistance in crossplane transport.

I note that the above discussion holds for acoustic phonons, which are the dominant carriers of heat in semiconductors. The role of optical phonons in bulk heat transport has recently been highlighted, Hatta et. al. (1985) but they are relatively minor contributors to bulk heat transport owing to the low occupation number and group velocity. It is also unclear how optical phonons behave when crossing boundaries, but it is likely that their transmission is highly suppressed because their existence hinges of good crystallinity.

Calculation of in-plane and cross-plane thermal conductivities

First, each layer's thermal conductivity is calculated in the same way as the bulk thermal conductivity of a material but with an additional scattering rate due to the presence of interfaces. The layer thermal conductivity obtained this way will already be lower than the bulk thermal conductivity of the same material. Second, the TBR is calculated using a transmission coefficient interpolated from the AMM and the DMM values.

The denominator in the expression is a correction factor introduced following the modified definition of temperature as the phonon distribution at the interface is far from equilibrium. The correction ensures that the TBR vanishes at a fictitious interface inside a material.

Application to thermal modeling of a quantum cascade laser.

The quantum cascade laser is a common application of III-V SLs. The active region of a QCL consists of tens of repeated stages, where each stage consists of tens of thin layers. Thermal modeling of such devices has always been challenging because of the great anisotropy in the thermal transport caused by the SL structure. It is difficult to accurately describe the in-plane and cross-plane thermal conductivity of such structures with existing simulation methods because of the complicated layer structure inside one stage. It is often assumed that the in-plane thermal conductivity of a SL structure is 75% of the corresponding bulk average in all temperature ranges. Under this assumption, a constant cross-plane thermal conductivity is used as a tunable parameter to fit the measured temperature profile. I show below that the assumption about the in-plane thermal conductivity being 75% of the weighted bulk value does not generally hold. This ratio is lower and temperature dependent, varying from 40% to 70% as the temperature rises from 100K to 400K. Overestimating the inplane leads to somewhat underestimating the cross-plane thermal conductivity based on a fit to a temperature profile.

RESULTS AND COMPARISON WITH EXPERIMENTS

GaAs/AlAs superlattices

I have compared the results from our simple model with several experimental results by different groups on both the in-plane Yao (1987), and cross-plane Yu et.al. (1995), thermal conductivity of III-arsenide SLs and obtained good agreement.

Conclusion

The presented model is fairly simple yet quite accurate, especially when used with full phonon dispersions. It can be very useful for thermal modeling complicated QCL structures, with many interfaces. The model is also applicable to other material systems where SLs have good-quality interfaces and phonon transport can be considered incoherent.

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INDUCTION OF GENETIC VARIATION IN AGRONOMIC TRAITS OF CHICKPEA USING MMS AND HZ

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ABSTRACT

An indispensablerequirement for any crop improvement programme is the availability genetic variation in the crop gene pool. In chickpea, exhausted genetic variability due to adaptation to various stresses through natural selection and conventional selection methods for homozygosis resulted into limited accessible genetic variability, and hence supplemented breeding strategies needs to be incorporated to serve the objective of crop improvement. In the present study, mutagenesis has been employed to investigate the comparative impact of HZ and MMS on economic traits of chickpea (*Cicer arietinum* L.) genotype (avrodhi) at M_2 generation. The assessment on polygenic expression of quantitative traits showed considerable deviations in all the treatments and significant positive shift at different doses compared to control. A broad spectrum and frequency of macro mutations were also induced affecting all plant parts and different morphological variants were studiedon the basis of economic importance from the treated populations. Economically important mutations like branching pattern, stem structure, plant height, dwarf and bushy growth habit, foliage type, flowering behavior and maturity were identified and the frequency of the variants were found to be more in HZ doses. Explicitly, MMS doses provided greater deviations in both directions in the quantitative phenotypic characters studied while frequencies of some distinct morphological variants were more in HZ. The induced elite phenotypes (blue flowered, double flowered, red pigmented leaf, bushy and early mutants), having strong correlation with agronomic traits, will definitely be helpful in selection of improved mutants in subsequent generations.

Keywords: Chickpea (*Cicer arietinum* L.), hydrazine hydrates (HZ), methylmethanesulphonate (MMS), mutation breeding, quantitative traits, morphological variation.

INTRODUCTION

Chickpea(*Cicer arietinum* L.), commonly called as Gram, is extensively cultivated as a rabi crop throughout India, especially in the northern states. Gram is considered to have originated in the tract lying between the caucuses and the Himalayas from where it spread into south Europe, Iran, Egypt, and India. The earliest record dates from about 4000 BC at Atranjikhera in Uttar Pradesh.

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There are two main groups of chickpea Desi (wrinkled seeded) which constitute about 85 percent of the total production and Kabuli (round seeded) which form the remaining 16 percent of the seed produced. In India generally cultivars with small to medium sized, brown wrinkled seed which are adapted to marginal growing conditions are grown while in western Asia the Kabuli type mainly grown as a spring sown crop. Average yield in India over the past two decades have fluctuated between 550 and 650 kg / hectare. The major target of the Indian chickpea improvement programmes is to produce cultivars which while being tolerant or resistant to some of the unstablizing factors, are also capable of higher yields under rain & irrigated conditions, and responsive to phosphatic nutrition. With these objectives the All India Co-Ordinate Pulse Improvement Project (AICPIP) has developed pulse multidisciplinary research programmes for chickpea improvement. Gram is an important source of dietary proteins, B-group vitamins and certain minerals extensively used as a protein adjunct to starchy diet. In India, agriculture gram occupies a unique position by virtue of its high protein content and its capacity for fixing atmospheric Nitrogen. It ranks 5th in area and 4th in production among the food grains in India.Among the grain legumes grown in India-gram ranks first with an annual average of 7.9 million hectare and production of 5.4 million tons. It contributes as high as 34.39 percent and 47.88 percent to the total area and production respectively of pulse in the country.

Chickpea being the third most important pulse crop in the world, substantial increase in the global yield has been the area of concern despite extensive breeding efforts (Gaur and Gour, 2002). An essential prerequisite for any crop improvement programme is the available genetic variation in the crop gene pool. The narrow genetic base of cultivated chickpea (*Cicer arietinum* L.), as detected from little polymorphism for isozyme, RFLP and RAPD markers (Gaur and Slinkard, 1990, Simon and Muehlbauer, 1997), is considered to be the major constraint in plant breeding for crop improvement. Therefore, to break this genetic bottle neck in Chickpea, additional breeding strategies is needed for achieving the objective of crop improvement. Micke (1988) and Yildirim*et al*., (2013) advocated the importance ofinduced mutations as one of the most effective and efficient approaches to regenerate and restore the genetic variability in chickpea. Legumes generally loose different alleles for high productivity, seed quality, pest and disease resistance during the processes of adaptation to environmental stress (Dhumal and Bolbhat, 2012). Mutation breeding is used to induce mutations at loci controlling economically important traits and/or eliminates undesirable genes from elite breeding lines (Lippert et al., 1964). Demand on mutation breeding to contribute to sustainable global food security and livelihood is increased tremendously in recent times. Several morphological mutants have been found and utilized in chickpea improvement as well as in linkage studies (Dahiya et al., 1984; Pundir and Reddy, 1998; McNeil et al., 2007; Kharkwal et al., 2010; Wani, 2011; Laskar et al., 2015).Considering the economic importance of *Cicer arietinum* L. and the limited work on induced mutagenesis through chemical mutagens the present work was undertaken to assess the possibility of induced variation and its impact.

MATERIALS AND METHODS

Dry (moisture content 10-12%) and healthy seeds of the chickpea genotype avrodhiprocured from the Government Seed Store, Aligarh, were used for mutagenic treatments of MMS and HZ. The seeds were first pre-soaked in distilled water for 9 hours and then directly transferred (25 seeds each) to the different concentrations of mutagens for 6 hours with intermittent shaking at room temperature of 25±2°C. To begin with, a pilot experiment was

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conducted to determine the suitable concentrations of the mutagens and duration of treatments for the crop using seed germination count and seedling height from the Petri plates kept in the BOD incubator at 27 ± 1°C temperatures. After that, the working solutions of MMS (0.01%, 0.02%, 0.03%, and 0.04%) and HZ (0.01%, 0.02%, 0.03%, and 0.04%) were prepared in phosphate buffer at pH 7.0 and pH 3.0 respectively. The pH of the solution was maintained by using buffer tablets (MERCK manufactures, Mumbai, India). After treatment, the seeds were thoroughly washed in running tap water for 30 minutes to remove the excess of mutagen. Five replicates of 5 seeds each were sown for each treatment along with untreated (control) in 9" earthen pots filled with a well prepared growth media of Farm Yard Manure, soil and sand with a ratio of 1:1:1 and kept in the net house of the Department of Botany, Aligarh Muslim University, Aligarh during the rabi season of the year 2013-14 to raise M_1 generation. The individually harvested seeds of normal looking M_1 plants per treatment were advanced for raising M_2 generation in the agricultural fields of Aligarh Muslim University, Aligarh, India from mid-October 2014 to April 2015. The experiment was designed in triplicate (50 seeds/replication) in three rows for each treatment following a complete randomized block design. Mean (X), Standard deviation (S.D.) and coefficient of variation (C.V.%) were calculated to determine the degree of intra and inter-population variation induced and statistical significant analysis were done using IBM SPSS statistics 20.

RESULTS

Differential response of the genotype was observed with respect to different doses of mutagens. Quantitative analysis of the treated plants showed wide range of significant phenotypic variations. (Table 2). The mutagenic effect of hydrazine hydrate (HZ) and methyl methane sulphonate (MMS) were studied on seed germination and percentage inhibition, seedling height and seedling injury, morphological changes in leaves and certain quantitative characters of *Cicer arietinum* L.The results are being presented below

Biological damages

The germination started a second day after sowing in control and in mutagen treated population of chickpea. Seed germination was found to be decreasing with increasing concentration of mutagens. In the control seed germination was recorded 96 % whereas it decreases from 88% in 0.01% MMS to 44% in 0.04%HZ. Data on seed germination was recorded in both pots as well as Petri plates (Table 1). The height of seedlings in Petri plates was measured in a separate experiment in addition to the pot experiment. The study of seedling height in Petri plates after 11 days of sowing showed that in control the seedling length measured was 26.23 cm. With the increasing concentration of MMS and HZ the seedling height decreased considerably. The maximum injury was observed in 0.03% MMS (20.52%) and 0.04% HZ (16.30%). (Table 1).

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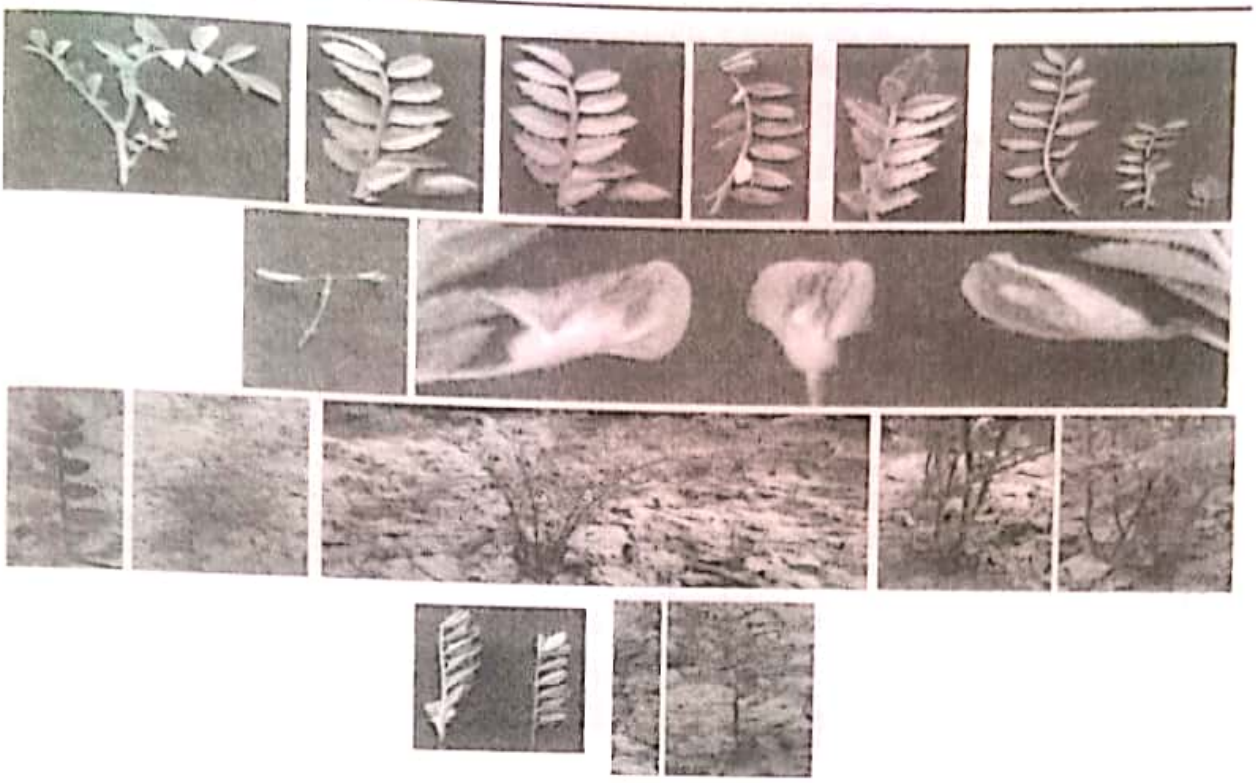

Figure 1: 1st raw showing different variations in leaf morphology and leaflet position. 2nd raw showing double flowering and flower colour range from whitish pink to bluish pink. 3rd raw showing plant growth habits, branching and pigmentation.

Quantitative traits

In control the plant height observed was 62.03 cm. In MMS treated plants the plant height increased initially with the increasing concentration of mutagen 0.01% MMS(64.37 cm) and 0.02% (64.26 cm) as compared to control while in HZ treated plants, the plant height showed highest positive shift from control with 0.01% HZ (65.38 cm) and 0.02% HZ (65.80 cm).However, at higher concentration of both the mutagens dwarf variants were observed, 0.03% and 0.04% of both MMS and HZ brought reduction in the plant height compared to control with highest in 0.04% HZ (39.72 cm) (Table 2).

Table 2: Statistical analysis of comparative effect of mutagens (HZ and MMS) on various quantitative phenotypic characters in M_2 generation of chickpea (*Cicer arietinum* L.) genotype avrodhi.

MUTAGEN DOSES	Plant length (cm)				Fertile branches per plant				Number of pods per plant				50 seeds weight (gm)			
	Mean±S.E.	S.D.	C.V.%	Shift in mean	Mean±SE,	S.D.	C.V.%	Shift in mean	Mean±SE	S.D.	C.V.%	Shift in mean	Mean±SE.	S.D.	C.V.%	Shift in mean
CONTROL	62.03±0.18d	1.31	2.11	--	13.32±0.02b	0.14	1.05	--	82.10±0.52d	1.14	1.38	--	14.77±0.115a	0.364	2.46	--
0.01% MMS	64.37±0.36bc	1.62	2.51	2.29	12.58±0.03c	0.15	1.19	-0.74	84.33±0.18c	1.15	1.36	2.23	10.55±0.110f	0.349	3.30	-4.222
0.02% MMS	64.26±0.07c	1.12	1.74	2.26	14.92±0.09a	0.26	1.74	1.60	85.20±0.17b	1.26	1.47	3.10	13.55±0.306cd	0.97	7.15	-1.222
0.03% MMS	50.48±0.14f	1.24	2.46	-11.55	11.55±0.08d	0.24	2.07	-1.77	86.43±0.37a	1.24	1.43	4.33	14.45±0.097ab	0.308	2.94	-0.332
0.04% MMS	43.85±0.22g	1.38	3.15	-18.18	11.34±0.03d	0.15	1.32	-1.98	82.00±0.20d	1.15	1.40	-0.10	14.19±0.128b	0.406	2.86	-0.588
0.01% HZ	65.38±0.84ab	1.46	2.23	3.35	12.97±0.01b	0.12	0.92	-0.35	84.10±0.17c	1.12	1.33	2.00	12.47±0.129e	0.408	3.27	-2.330
0.02% HZ	65.80±0.18a	1.31	2.00	3.77	8.60±0.06e	0.21	2.44	-4.72	86.43±0.28a	1.2	1.38	4.33	13.81±0.108c	0.342	2.47	-0.966
0.03% HZ	56.27±0.12e	1.21	2.15	-5.76	11.52±0.07d	0.22	1.90	-1.80	86.40±0.25a	1.22	1.41	4.30	13.77±0.411c	1.30	9.44	-1.000
0.04% HZ	39.72±0.26h	1.46	3.68	-22.31	6.21±0.35f	0.61	9.82	-7.11	80.43±0.27e	1.71	2.12	-1.67	13.30±0.151d	0.480	3.60	-1.477

Means within columns followed by the same letter is not different at the 1% level of significance, based on the Duncan Multiple Range Test.

The number of fertile branches was counted after the flowering in the plant. In control, the number of fertile branches observed was four (13.32). In MMS treated plant, it ranged from 14.92 to 11.34 and in HZ treated plants; it ranged from 12.97 to 6.21. The maximum number of fertile branches was observed in 0.02% MMS while minimum 0.04% HZ (Table 2).

Number of pods per plant was counted at the time of maturity in plants. In control the mean number of pods per plant were observed as 82.10 while in MMS treated plants the number of pods per plant ranged from 86.43 to 82.00, with highest in 0.03% MMS while in HZ treated

plants the number of pods per plant ranged between 86.43 to 80.43, with highest in 0.02% HZ. Lowest number of pods counted in 0.04% HZ (80.43) (Table 2).

Weight of 50 seeds per plant was performed at the time of harvesting of plants. In control the average weight of 50 seeds per plant was 14.77 gm, while in MMS treated plants the seed weight ranged between 14.45 to 10.55 gm, the maximum seed weight per plant was observed in 0.03% MMS concentration (14.45) while in HZ treated plants the seed weight ranged between 12.47-13.81, the maximum seed weight per plant was observed in 0.02% concentration (13.81gm).

The results of the present pursuit showed that lower and moderate doses of the chemical mutagens could induce useful quantitative phenotypic mutations in chickpea for screening and selection purposes. Background reasons for these mutations could be induce growth stimulation by changing the hormonal signaling network or by increased anti-oxidative capacity of the cells due to mutagenesis. Explicitly, stimulation of growth and improved immunity to daily stress factors due to mutagenesis definitely resulted into expression of desirable quantitative characters in chickpea plants.

DISCUSSION

In plant breeding, generally the criteria such as germination, injury, lethality, sterility and chromosomal aberrations are used to assess the superiority of mutagens and desirability of mutants' genotypes. Mutations affecting growth habit, flower color and plant type have been reported in chickpea earlier (Ahmad and Godward 1993, Kharkwal 1999, Gaur and Gour 2001). The results in the previous section showed that the plants were more sensitive to the HZ mutagen than MMS. Methyl methane sulphonatemethylase DNA on N^7- deoxy guanine and N^3-deoxyadeneine. The data collected showed that seed germination was reduced with the increasing concentration of both the mutagens. Reduction in seed germination has been explained due to delay or inhibition in physiological and biological processes necessary for seed germination which include hormonal imbalance (Ananthaswamy*et al.*, 1971). Germination as well as emergence can be drastically reduced due to imbibitional chilling injury and consequent infection by soil borne pathogens (Chen *et al.*, 1983, Balasubramanian*et al.*, 1998). Seedling heights were also affected considerably at different doses of both the mutagens used. It was observed that there is a great deal of variation for root and shoot growth characters in chickpea mutants (Cancie*t al.*, 2004).The result showed that the plant height was increased in both mutagens treated populations as compared to control meaning that the increasing concentration showed a stimulatory effect at initial concentrations. But at some higher concentrations of the mutagens dwarf plant were observed. Similar results of wide occurrence of dwarf mutants in chickpea were also reported by Khan *et al.*, 2011. Dwarfs may be due to reduced internode length or internode number or both (Sjodin 1971). In our study reduction in internode length was mainly responsible for dwarfness. Different reports are available to explain the reduction in seedling growth. Gray and Scholes (1951) suggested that it could be due to genetic injury in meristematic cells. Goud and Nayar (1968) demonstrated that the depression in seedling growth may be due to the inhibition of auxin synthesis. Morphological variants, bushy plant type with excessive branching and increased number of inflorescence, slightly grooved surface of primary shoots, narrow leaves were investigated and compared to control. In chickpea, erect or bushy growth habit is a characteristic controlled by a single gene (Hg/hg) where erectness is dominant and bushy is recessive Khan *et al.*, 2011. Variations in leaf morphology and chlorophyll variants

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were also observed at higher concentrations of mutagens. Number of pods per plant showed inhibitory effect in HZ treated plants with increasing concentration while MMS treated plants showed both stimulatory and inhibitory effect. The increase in yield contributing characters like pods per plant was reported in *Viciafaba* (Ismail *et al.*, 1977), *Cicer arietinum* (Sharma *et al.*, 1990) while the decrease in the number of pods was reported by (Amer and Farah 1976) by carbamate pesticide. These reports show that mutagenesis is a potential tool to be employed for crop improvement. The decrease or increase in the number of pods occurred due to induced mutation in the meiotic cycle which affected the frequency of normal microspores up to a greater extent and the megaspore to a lesser extent and hence the fruit set was directly affected. Various chromosomal abnormalities are related to pollen fertility and ultimately the seed set. Weight of 50 seeds was decreased with increasing concentration in MMS treated plant, while in HZ treated plants it showed variation as compared to control. The earlier works showed that increase in 100 seed weight was observed in *Oryza sativa*and decrease in 100 seed weight was observed in *Capsicum annum* by the herbicide. The increase and decrease in 100 seed weight occurred due induced mutation in the meiotic cycle which affected the frequency of normal microspore and hence the fruit set was directly affected.

SUMMARY AND CONCLUSSION

It has been concluded from the combined analysis of the different parameters considered in the present experiment, that MMS is more effective in inducing variation compare to HZ in *Cicer arietinum*.The morphological variation observed in the present investigation is due to physiological, biochemical; metabolic and genic disturbances induced by the action of chemical mutagens used in the present study (MMS and HZ) and might be also due to their interaction with environment. The effects of chemical mutagens (MMS and HZ) used in the present investigation for induction of variation in *Cicer arietinum* L. have been studied in $M_1$and M_2generation and further investigation of M_2 mutants inM_3 generation will certainly provide some superior mutant line, which can be directly used in the selection of new variants and also as an initial material for the hybridization program in plant breeding. Since, Chickpea (*Cicer arietinum* L.) is an autogenous crop with natural cross pollination ranging between 0–1%; therefore, there is a lack of sufficient natural variation. Thus, the induce mutation is the only efficient tool to create new genetic variability in chickpea. Improvement of crop productivity is becoming ever more important in view of population growth on one hand, and the lack of new arable land to bring into cultivation, water for irrigation and climatic change on the other hand. Thus, demand on mutation breeding to contribute to sustainable global food security and livelihood is increased tremendously in recent times. Mutation breeding is the lasting hope to generate genetic variability needed to enable our food crops to adapt to changing environments.

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