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NEMATODES IN THE SALINE ENVIRONMENT: A MINI OVERVIEW

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ABSTRACT

The ecology of free-living nematodes in various natural salinity-affected habitats (viz. Mc Murdo and Taylor valleys of Antarctica, estuaries, deep Sea, Aral Sea bottom, halophytic tuft grass nematodes of golf courses, hyper-arid and hyper-saline oasis and desert soils, coastal entomopathogenic nematodes, stylet-bearing nematodes of mangroves of Sundarban and salinity relations of parasitic nematodes in agricultural systems (hatching of their eggs, larval survival, and infectivity of host plants, as studied by various nematologists, are briefly overviewed.

Key-words: Salinity, free-living, entomopathogenic and parasitic nematodes, survival, hatching, infectivity of host plants

INTRODUCTION

Nematodes are the most numerous of all the multicellular organisms (Platt *et al.,* 1984). Almost 500,000 species of nematodes are known world wide (Hyman, 1951). They occur in every habitat which can support life. Nematodes depend on the water films around soil or organic material and more within existing pathways of soil pores of 25 to 100 µm diameter (Neher, 2020). They are the most diverse among marine metazoan taxa (Platt and Warwick, 1980). The free living nematodes of aquatic environments although highly abundant, often numbering millions per m^2, remained relatively unstudied (Heip *et al.,* 1985). They are small but very important in the ecology of seas and rivers and agricultural ecosystems. In benthic environment nematodes are important from energy-flow-viewpoint in the ecosystem being significant part of diet of many aquatic organisms (Gee, 1989), and facilitating the mineralization of organic matter (Riera and Hubas, 2003).

Vast area of land is salinity-affected as a result of poor soil drainage, improper irrigation methods, poor water quality, and insufficient water supply for adequate leaching (Backland and Hoppes, 1984). Salinity is one of the important factors which restricts economical utilization of land resources of inland and coastal areas of the World (Maas, 1986). It is estimated that salinity seriously limits crop production on 20 Million hectares (Al-Ashry *et al.,* 1985). High evaporation rates of plants in arid regions may lower soil matric potential and increase salt concentration around roots. The result is the fluctuating osmotic and ionic effects on organisms in or around the root zone (Edongali and Ferris, 1982). Such soils contain variable amounts of cations and anions at different concentrations and complexities. The common dominant anions are Cl^-, $SO4^{2-}$, HCO_3^-; sometimes NO_3^- also. Na^+, Ca^{2+}, Mg^{2+}, and K^+ are common cations. These ions may exist individually or in combination with others to form complex compounds. The concentration of the salts in the soil is expressed as $dS.m^{-1}$ (deci-siemen per meter). It is estimated that water or soil extract containing 640 $mg.L^{-1}$ total dissolved salts has an electrical conductivity (EC) of c. $1dS.m^{-1}$ or a molar concentration of c. 11 millimol (mM) NaCl. A saline soil is one with EC of 4 $dS.m^{-1}$ or greater (Anon., 1984).

Salinity as well as nematodes, both is important in agriculture. Salinity affects the plant, which is a host to so many nematodes. The chemical composition of soil solution directly affects nematodes (Bird, 1977). A great deal of work has been done on salinity relations of plants. The influence of salts on nematode infectivity is not much clear. The hatching of eggs, parasitism and various developmental stages are likely to be affected by the salinity. The overall effects probably vary depending upon the combination of nematode, host, chemical causing salinity and level of salinity, linked with other factors in soil (Khan and Khan, 1990). The role of salinity in nematode-plant interaction has been partially studied in cotton (Heald and Heilman, 1971), tomato (Maggenti and Herdman, 1973), sweet orange (Van Gundy and Martin, 1961), Okra and brinjal (Jain *et al.,* 1989). Besides nematode ecology in natural saline and hyper-saline ecosystems which supports a huge number of free-living nematodes, this paper also briefly explores the effects of salinity on nematode survival, their hatching and behaviour and plant-nematode interaction..

A. FREE-LIVING NEMATODES IN SALINE ENVIRONMENT

1. Nematodes of Bull Pass Mc Murdo Valley, Antarctica

McMurdo dry valley (Antarctica) is a hyper-arid polar desert with low precipitation and soil temperatures. It undergoes brief periods when moisture availability and temperatures are suitable for biological activity. There are two nematode species in Bull Pass Mc Murdo dry valley with the dominant *Scottnema lindsayae* occurring in substantial number of the samples and *Plectus antarcticus*. There were negative correlations between live nematode abundance and soil nitrate concentration and salinity (Poage *et al.,* 2008). Harsh environment studies have indicated that in this environment salinity may be more important than soil moisture in explaining the abundance of community structure of soil nematodes (Barrett *et al.,* 2004; Courtright *et al.,* 2001; Nkem *et al.,* 2006). Salt solutions ranging from 0.1 to 3M and saturated soil extract from several of McMurdo dry valley ECe ranged from 0.11 to 12 $dS.m^{-1}$ (Nkem *et al.,* 2006). Nematode salt tolerance was found to be species specific. Two abundant species of nematodes were *Scottnema lindsayae* (most abundant) and *Plectus antarcticus* (from the Taylor valley). Both species survived low NaCl and $MgSO_4$ concentrations. Neither species survived in KNO3 solutions of any concentrations. There was survival in salinity of 4.1 $dS.m^{-1}$. There was 80-97% survival in low salinity of 1.95 $dS.m^{-1}$. A 1:1 dilution of highly saline saturated paste extract increased *S. lindsayae* survival to 80%; while survival of *P. antarcticus* was not observed until dilutions of greater than 200%. The results demonstrated niche partitioning of *S. lindsayae* and *P. antarcticus* (Nkem *et al.,* 2006).

Treonis and Wall (2005) suggested that coiled morphology of nematodes obtained from dry soils of Taylor valley, Antarctica employ anhydrobiosis and they showed enhanced revival when rehydrated in water as compared to vermiform nematodes. Nematode coiling was correlated with soil moisture content, salinity and water potential. In austral summer, the Taylor valley's coiled nematodes proportion vary diurnally – with more nematodes vermiform presumably active at the warmest time of the day. Dry valley nematodes uncoiled rapidly in response to soil wetting from snowmelt. Anhydrobiosis represents an important temporal component of a dry valley's nematode life and probably attributes to their widespread occurrence and success in this extreme environment.

2. Estuarine Nematodes

The habitat of mud-dwelling estuarine nematodes consists of essentially an extremely complex mixture of inorganic and organic particles surrounded by interstitial water (Baver, 1948). Ecological factors here are quite peculiar and very much different from those affecting nematodes living on rocky shores, intertidal sea weeds or marine sandy beaches (Capsrtick, 1959). Most important variable is salinity of the interstitial water. Rees (1940) has shown that nematodes in intertidal mud have their largest population densities in the surface 1 cm and as the depth of mud increases the population densities decrease until at a depth of 5 cm few nematodes are found. The distribution of free living nematodes were earlier studied under varying salinities in the Baltic, Belgian coast, North seacoast of Germany, Dutch coast and reviewed by Wieser (1951). Capstick (1959) described densities of 37 nematode species in different reaches of the River Blyth estuary - of these the four species viz. *Spirina parasitophora*, *Sabateria* sp. *Anaplostoma viviparum* and *hyplodontolaimus zosterae* were the most abundant species. A total of 27 genera of Nematoda were recorded in the Tramandai-Armazem estuary on the North coast of Rio Grande do Sui, Brazil. The highest number of organisms occurred in the summer. An interaction of salinity, hydrostatics, availability of food and the temperature influenced the community structure (Kapusta *et al.,* 2006).

The species composition of nematodes in the microtidal estuaries of temperate regions in the Northern Hemisphere has been demonstrated as the function of the salinity and the characteristics of the sediment (Warwick, 1971; Austen and Warwick, 1989; Soetaert *et al.,* 1995). In their studies Hodda and Nicholas (1985; 1986) found that in the intertidal zone of the mangrove-dominated Hunter River Estuary in temperate eastern Australia the densities of nematodes correlated most closely with elevation above low tide mark and pollution levels but neither with salinity nor the median grain size. Hodda (1990) attributed those variations of nematode fauna at this estuary and other two adjacent estuaries more related to Oxygen penetration, organic content and grain size composition. The Swan River estuary in south-western Australia has been comprehensively studied by Hourston *et al.* (2009). They found *Spirinia parasitifera*, *Theristus* sp., *Comesoma arenae*, *Bathylaimus australis*, *Chromodorina* sp. *Dichromodora* sp, and *Viscosia glabra* to be the important species of this estuary and a new sp. *Paradontophora aurata*. Furthermore, the spatial distribution of the composition of the nematode assemblages was found to be closely related with salinity and, to a lesser extent, grain size composition and amount of particulate organic material in the sediment. Alongi (1990) concluded that physical factors (temperature, grain size, salinity, in frequent tidal inundation limiting dispersal) accounted for the differences observed rather than biological variables. Shabdin and

Othman (2008) found that Pearson correlation 'r' demonstrated that the species diversity and density of the nematodes were influenced by the height of the beach. Clustering and detrended correspondence analyses did not clearly showed such effects. Shabdin and Othman (2008), therefore, concluded that there were no universal causative factors which control the horizontal distribution of free-living nematodes in the sandy and muddy habitats of the Lok Kawi Beach, Malaysia. Gingold *et al.* (2010) have viewed the situation in terms of environmental heterogeneity. The nematofauna of structurally complex beach, they reported, to be more diverse than the one from a homogeneous beach nearby – this supports the hypothesis that structural heterogeneity promotes diversity by compartmentalization and highlighting the importance of the microhabitats in the assessment of biodiversity (Gingold *et al.,* 2010).

Coastal muds are characterized by a few dominant genera which all belong to families Comesomatidae, Linnomoeidae, Xyalidae, Spiriniidae and Sphaerolaimidae. This assemblage seems to occurring world wide indicating existence of parallel communities (Heip *et al.,* 1985). Based on the studies of Fell *et al.* (1975) and Krishnamurthy *et al.* (1984) in India and Shabdin and Othman (2008) in Malaysia it is apparent that predatory nematodes are more abundant in the tropics than in the other areas.

Subtidal nematode community along salinity gradient in Southern European estuaries (two Portuguese estuaries) have been investigated by Adão, *et al.,* (2009). According to the Venice System of salinity classification these estuaries were characterized by i) Fresh water and oligohaline (0.5 - 5‰ salts) areas, ii) Mesohaline (5 -18 ‰ salts) areas, and iii) polyhaline to euhaline (18 – 30‰ 0r > 30 ‰ salts) areas. The communities of these estuaries (Mira and Mondego) closely resembled to Northern European estuaries. *Oxystomina* and *Prochromatorella* were exclusive to oligohaline zone; *Terchellingia*, *Sabatieria* and *Daphtonemia* were found in Mesohaline zone and *Paracomesoma*, *Synonchiela* and *Odontophora* in the polyhaline and euhaline zone.

The diversity of nematodes is low in euhaline to polyhaline zones, reaches to a peak in polyhaline to Mesohaline and decline in the Mesohaline to oligohaline zones. The diversity is generally low in sandy sediments at the mouth of estuaries probably due to turbulence and periodic re-working of the sediments (Heip *et al.,* 1979). The species composition of nematode communities in estuarine environment is a function of salinity and the sand grain size (Heip et al., 1985).

Moens and Vincx (2000) have studies the life cycle of *Pellioditis marina* and *Diplolaimelloides meyli* isolated from Mesohaline zone of Westerschelde Estuary SW Netherlands. Salinity had relatively minor effects on fecundity, development times and sex ratios in both species but strongly impacted on juvenile viability at the extremes of the salinity range: salinity close to 0 and 4.0%; pre-adult mortality was more than 80% in *P. marina*. It was 100% at 5.0 % in *D. meyli*. Both had optimal fitness at salinity between 1.0 to 3.0%. The development was slow at 10^{o}C and no reproduction took place at temperature below 10 ^{o}C. Female biased ratio was found in D. meyli at low temperatures and in *P. marina* under optimal salinity conditions.

3. Nematodes of Deep Sea and Aral Sea Bottom Water

An account of nematodes and their densities in deep sea communities studied in NW Atlantic, North Sea, W. Mediterranean, W. Pacific, Ibernian deep sea and Venezuela Basin has been compiled by Heip *et al*., (1985). The density generally ranged from a few to 1500 individuals per cm2 except in NE Atlantic (Faroës) to be high (3999) as reported by Thiel (1971).

In Aral Sea in 2003 and 2004, from water depth ranging from 0 to 39m and near bottom water salinity at the sampling sites varying from 88 to 109 ‰ salinity; maximum abundance of free-living nematode was recorded to be 1440 specimens / 10 cm^2 in 2003 and at a depth of 10m at the site salinity of 88.9 ‰ and water temperature of 13.6 ^{o}C. In 2004, maximum abundance was 750 specimens / 10 cm^2 at about the same salinity and depth but temperature, 24.5^{o} C (Mokievsky, 2009).

4. Nematodes of halophytic Tuft Grass of a Golf Course

Paspalum vaginatum, Seashore Paspalum is a warm season tuft grass adapted for saline conditions and used on golf courses. Sting nematode, *Belonolaimus longicaudatus* and lance nematode, *Haplolaimus galeatus*, both are parasitic and injurious to *Paspalum*. *B. longicaudatus* is ectoparasite and damages lateral roots as they emerge and *H. galeatus* is endoparasitic and migratory and damages by feeding and tunneling through cell walls. Both decrease root growth and above growth manifestations in sporadic turf thinning and chlorosis. After 120 days of salinity treatment final population of *H. galeatus* was reported by Hixson *et al.,* (2005a) to be negatively linearly correlated with salinity (0 – 16,000 ppm TDS) in 2002 and 2003, both years. In *B. longicaudatus* such a relationship within the given range of salinity was curvilinearly defined by log-transformed final population in 2002 and 2003. The optimum salinity was 9,600 ppm TDS.

Haplolaimus galeatus
2002 – Log Population = - 0.07 X + 2.34, R^2 = 0.92
2003 – Log Population = - 0.05 X + 2.82, R^2 = 0.83

Belonolaimus longicaudatus
2002 – Log Population = 0.007 X2 + 0.12X + 1.80, R^2 = 0.72
2003 – Log Population = 0.006 X^2 + 0.10X + 2.38, R^2 = 0.78
Here X = TDS (ppm) in the irrigation water

Hixson *et al.,* (2005a and b) concluded that the irrigation with pure Seawater or blended highly saline Seawater have to be option to suppress lance and sting nematodes. It is the vital information for tuft grass managers maintaining Seashore Paspalum where nematode problem do arise. Saline irrigation affects the both organisms significantly. The comparison of root length of *Paspalum* revealed that *B. longicaudatus* caused root stunting at low salinity (0 -10dS.m^{-1}) but roots were not affected at 15-25 dS.m^{-1} which indicates that the ability of *B. longicaudatus* feed and stunt root growth was negatively affected by salinity level of 15 dS.m^{-1}.

Seven commercial cultivars of sea shore *Paspalum* have been tested under green house condition by growing in sand-filled plastic containers inoculated with sting (50 / pot) or spiral nematodes (500 / pot) Cultivars 'Salam', 'Sea-dwarf', 'Sealsle Supreme', were the most tolerant to sting nematode and 'Seaspray' and Sealsle" were tolerant to spiral nematode. No cultivars were tolerant to both nematodes (Pang *et al.,* 2011).

6. Salinity Relations of Some Entomopathogenic Nematodes

Heterorhabditis bacteriophora and *Steinernema gleaseri* are entomopathogenic and seek out host insects (*Galleria mellonella* larvae) actively. Under salinity of 0 – 30dS.m^{-1}, survival, penetration efficiency or movement of *H. bacteriophora* through a soil column were not influenced by KCl. NaCl above 16 dS.m^{-1}, however, adversely significantly. These parameters of survival, virulence and penetration of *S. gleaseri* were not affected by KCl, NaCl and $CaCl_2$. High concentration of salts inhibited *H. bacteriophora* ability to move through the soil column and locate its host insect larvae and infect it. Larvae of the nematodes employed in this study were reared in *Galleria mellonella* larvae (Thurston *et al.,* 1994).

Another species of *Steinernema, S. carpocapsae* infectivity to *G. mellonella* larvae was found to be unaffected by NaCl concentration up to 16 dS.m^{-1} (Oetting and Latimer, 1991). Das (1977) demonstrated that *S. carpocapsae* could survive salinity levels up to 20 dS.m^{-1}. Mermithid entomopathogenic nematode, *Ramanomermis culicovorax*, was, however, poor in survival and infectivity at 4.8 dS.m^{-1} NaCl (Brown and Platzer, 1978). The use of *Steinernema* and *Heterorhabditis* is reported to be more suitable in saline soils in field conditions (Thurston *et al.,* 1994) in control of *Galleria mellonella*.

Several species of entomopathogenic nematodes of genera *Heterorhabditis* and *Steinernema* were isolated by Shahina and Maqbool (1996) from various climatic regions of Pakistan. Several isolates of these nematodes were tested for salinity and temperature tolerance (Shahina *et al.*, 2005). *Heterorhabditis* spp. are frequently found in coastal sandy soils. *H. indica* (isolate 7 ma) is reported from Karachi. It has been reported to survive in sea water only at higher temperature (40 ^{o}C).

7. Nematodes in an Arid Desert Oasis

Three habitats viz. sand dunes, riparian zone and saline meadows dominated by *Tamarix ramosissima* in an arid desert oasis in North-West China were studied by Yang-Zhong *et al* (2012) for various soil and nematode characteristics. The nematode abundance varied from habitat to habitat. Nematodes were highly abundant in sand dunes and the lowest in saline meadows. The soil under *Tamarix* community canopy had nematode in higher abundance and lower trophic diversity than in the soil of the open interspaces. Under the canopy of vegetation of sand dune bacteriophora increased and fungi ores decreased. Bacteriophores also pre-dominated in riparian habitat. It was further asserted that that improved soil fertility cannot limit the impact of Salinization beneath *Tamarix*. It should be take into consideration when using *Tamarix* as vegetative restoration plant because of adverse effects of salt accumulation on the soil environment.

Soil biota in Arava valley of Negev desert, Israel has been studied by Pen-Mouratov *et al.,* (2010). The physical and chemical characteristic of this hyper-saline area were exttrem3ely unfavourable for soil nematode communities and nematodes were entirely absent in the open area below sea level. The bacteriovores nematodes were found to be the most resistant in the hyper-arid region. with the *Wilsonema* being the most widespread genus.

8. Pollution Relations of Nematofauna

Nematodes are sensitive to pollution. Salinity, heavy metal content, organic carbon and hydrocarbon contents of sediments are the key factors negatively influencing the density, biomass and diversity of the nematofauna Mahmoudi *et al.,* 2002a). Significant temporal and spatial variation in mean meiofauna density in Goa India), in response to organic discharge via sewage and prevailing environmental conditions is reported by Ansari *et al.,* (1984). Total abundance, diversity and species richness of nematofauna communities is reported to decrease significantly with increasing levels of organic enrichment (Schratzberger and Warwick, 1998). Hydrocarbon content in Bay of Bizerta (Tunisia) is also reported to adversely influence free-living nematode biomass and diversity (Beyrem and Aissa, 2000). In Asghar EL Melh lagoon of Tunisia, Mahmoudi *et al.,* (2002b) reported that salinity and dissolved O_2 in water and the NH_3 content of sediments affects density, biomass and diversity of nematofauna.

There are certain characteristics of nematodes which suggest their use as bio-indicators. They have delicate permeable cuticle; they undergo crypytobiosis or cyst formation under adverse environmental state (many Dorylaimidae) have no resistant structure and are sensitive to environmental changes; they register heat shock and produce proteins conservatively, expression of which is enhanced under stress such as metal ions, organic toxins. These proteins could serve as bio-markers or eco-toxicological assessment of the soil (Ŝála and Rágala, 1984; Bongers, 1999; Hashmi *et al.,* 1997; Kammenga *et al.,* 1998, 2000; Neher, 2001; Geetanjali *et al.,* 2002).

9. Salinity Relations of Stylet-Bearing Nematodes Parasitizing Mangroves Roots

In the intertidal mud flats at Harinbari and Gangasagar of Sagar island mud flat of Prentice Island, there are large mangrove forests. Sinha and Choudhury (1988) reported highly salt tolerant stylet-bearing nematodes parasitizing roots of these mangroves. In the mud flat of Harinbari salinity is c. 2.8%, habitat is sheltered and dominated by mangroves *Acanthus ilicifolius* L., *Exocoecaria agallochia* L., and *Bruguiera gymnorhyza* (L.) Lam. With associated nematodes – *Dorylaimoides* sp. *Timmus* sp., *Nygolaimoides* sp., *Paradoxydirus* sp., *Hemicriconemoides sundarbanensis*, *Nothocriconema* sp. and *Thonus* sp. In intertidal sand flat (exposed habitat) *Phoenix peludosa*, *Suaeda maritima* Dumort, and *Sonneratia apetala* Ham. are the plant species associated with nematodes such as *Dorylliun aestuarii*, *Laimydorus parabastiani*, *Nygolaimus* sp., *Nygellus* sp., *Helichotylenchus* sp., *and Hirschmannialia gracilis*. The mud flat of Prentice Island has salinity c 2.8% and the dominant mangrove species are *Avicennia officinalis* L., *A. marina* Vierh. and *Ceriops decandra* (Griffith) with parasitizing nematodes – *Proleptonchus paucipappilatus*, *Indoditylenchus sundarbanensis* and *Tylenchu*s sp.. A habitat preference by the nematodes is clearly seen in their data.

B. SALINITY RELATIONS OF PLANT- PARASITIC NEMATODES

1. Salinity-induced Plant Symptoms

Plants adversely affected by salinity, grow slowly and become stunted. Leaves are smaller and thicker than those of normal plants. Chloride ion increases the elongation of the palisade cells, causing increased succulence (Strogonov, 1962). The leaves of salt-affected plants are often darker green than those of normal plants, but in some species (e.g., crucifers and some grasses) thick layer of surface wax cause a bluish-green cast. The stunting of fruits as well as leaves and stems occur (Bernstein, 1959, 1964). Plants growing under salinity may show no distinctive symptoms and only comparison with normal plants reveals the extent of salt-induced inhibition (Bernstein, 1975).

Lamb and Horne (1963) described *Rotylenchulus reniformis* infected cotton to be dwarfed, chlorotic with fewer secondary roots showing greater mortality among infected young plants. High soil salinity causes cotton plants to become stunted and their leaves to turn a dark green colour (Myers *et al.,* 1963). The symptoms of nematode injury and soil salinity in cotton are somewhat similar. Heald and Heilman (1971) noted that nematode inoculated plants of cotton at high salt levels dropped several bottom leaves and became very weak with thin stems. Similarly, at higher salinities okra and brinjal plants, irrespective of nematode infection were more stunted with smaller roots and shoot than in healthy plants (Jain *et al.,* 1989). Nematode damage is generally more evident at higher salinity (Edongali and Ferris, 1982).

In the presence of nematodes, salt stressed tomato plants were significantly smaller than non-infected plants. At higher salinity levels, tomato plants were stunted with succulent dark green leaves, resulting in the reduction of fresh and dry weight of the shoot compared to the plants growing in non-saline conditions. Nematode injury was generally more evident at high salinity where conditions were unfavourable for plant growth (Edongali and Ferris, 1982); Heald and Heilman, 1971; Maggenti and Herdman, 1973; Van Gundy and Martin, 1961).

2. Survival Movement and Behaviour of Nematodes under Salinity

Caenorhabditis elegans is the most thoroughly studied nematode. It is the only animal with an entirely known cell lineage from zygote to all 810 cells in the somatic tissues of the adult (Sulstron and Hodgkin, 1988) and completely mapped nervous system (wood, 1988). Williams and Dusenbery (1988, 1990) first proposed to use this animal for aquatic toxicity testing and several toxicological bioassays have been developed using this animal since 1990s.

Khanna *et al.* (1997) investigated tolerance of *C. elegans* to pH, salinity and hardness of aquatic media. The tests were run under two conditions, K medium and moderately hard re-constituted water (MHRW). Hardness tolerance was tested by adding $NaHCO_3$. *C. elegans* exhibited great versatility to the test conditions. Up to 15.46g.L^{-1} NaCl and 11.51 g.L^{-1} did not show any adverse effects compared to the control in K medium. Higher salt concentrations were tolerated in MHRW medium. About 20.5 g.L^{-1} NaCl and 18.85 g.L^{-1} KCl did not show any adverse effects. Nematode could tolerate 0.236 to 0.246 g.L^{-1} of $NaHCO_3$.

Tietjen and Lee (1972) have determined the salt tolerance of *Monhystera denticulata* Timm.(isolated from *Zostera marina* from North Sea Harbor, Southampton) under laboratory conditions at various salinity and temperature conditions. The optimal salinity and temperature condition was 2.6 % salts and 25 ^oC. Decrease in temperature of 10 ^oC and increase or decrease of salinity of 13 % resulted in doubling of generation time. At optimum conditions the generation time was 10-12 days.

2.1. Survival

The survival of nematode larvae may be influenced by various salts and ions. During dry and wet period, soil nematodes are subjected to variable salt concentration in the soil solution. The effect of different salts have not been recorded the same on the survival of different species of plant parasitic nematodes.

Ray and Das (1980) reported from India that many species of *Tylenchus, Tylenchorhynchus, pratylenchus* and *Aphelenchoides* were tolerant to slightly saline soil conditions, while species like *Helicotylenchus dihystera, Hirschmanniella gracilis* and *Macroposthenia ornata* thrived well in moderately saline soil. A few other like *Rotylenchulus reniformis, Hemicriconemoides spherocephala, Hemicriconmoides cocophillus* and *Caloosis exilis* tolerated strongly saline conditions. Among dorylaims; *Xiphimema vulgare, X. elongatum, Paralongidorus citri* and a few species of *Dorlaimus* and *Tylencholaimus* were abundant in saline soil.

In another report from India, Lal and Yadav (1976) found that populations of *Aphelenchus avenae, pratylenchus thornei, Helicutylenchus* spp., and *Rotylenchulus reniformis* in pot soil decreased at salt concentration of 40, 80 and 120 meq / L but the members of *Heterrodera avenae, Tylenchulus mashoodi* and nematodes which feed on bacterial and fungal spores were unchanged. Total nematode populations were significantly smaller at salt concentrations of 40 meq /L and above. Heald and Heilman (1971) reported that in a field with a large saline area, *Totylenchulus reniformis* and *Meliodogyne incognita* were found together. The reniform nematode distribution was aggregated in this field, but the nematodes were apparently not influenced by the degree of soil salinity. The numbers of Meliodogyne females per gram of root were significantly greater in the soil with an EC_e of 2 and 6 dS.m^{-1} than in those with EC_e of 12 and 18 dS.m^{-1}. These differences were apparently due to limitation of the development of the root system by the salinity and not to the effect of the salinity on the nematodes.

Kirkpatrick and Van Gundy (1966) found no significant change in a population of Citrus nematode (*Tylenchulus semipenetrans*) larvae inoculated in fallow saline soil after 184 days. However, larvae were significantly reduced at the salinity of 26 dS.m^{-1} after 68 and 184 days. Van Gundy and martin (1961) found that the effect of the citrus nematodes was usually most severe under soil conditions that were unfavourable for the growth of citrus. Under field conditions, Machmer (1958) concluded that more citrus nematodes were recovered from citrus roots subjected to high salinity levels. Maggenti and Herdman (1973) reported that with increasing salinity, the population level of *Meliodogyne javanica* initially dropped to less than half. Beyond that salinity level, the population remained at almost asymptote. The initial drop in nematode population and then maintenance of almost constant population at higher level of salinity may reflect larval survival rather than a lowering of the reproduction potential. According to Jairajpuri *et al.* (1974) various mineral salts show different effects at different concentrations; in general, the rate of survival is low at high concentrations. They found that copper sulphate was the most toxic salt in which only *Helicotylenchus indicus* survived for a maximum of ½ hour and the other three species viz. *Hoplolaimus indicus, Xiphinema basiri* and *Mylenchulus minor* died immediately upon introduction to this solution. At the higher concentrations of 0.4 to 0.2 M of copper sulphate, *Xiphinema basiri* and *Mylonchulus* *monor* survived for about a minute, but *Hoplolaimus indicus* and *Helicotylenchus indicus* survived for 6-7 minutes. In other salts (sodium nitrate, potassium chloride, and potassium carbonate) at 0.025 M concentration all these four nematode species survived for several hours. They also observed that potassium chloride is the least toxic of all the salts tested. Ramana *et al.* (1975) studied shrinkage and mortality in *Hoplolaimus indicus* in hypertonic solutions of NaCl in water (1 to 100 g / L) at interval a till complete kill was obtained. At 100g /L complete mortality resulted in 8 min; at 10g/L, shrinkage and mortality occurred in 195 min.

Robinson *et al.* (1984) conducted an experiment in which survival of *Orrina phyllobia* (J4) was examined in sodium chloride and synthetic soil solution that contained Na, ca, Mg, Cl, and NO_3 ions at relative concentrations identical to those in a known agronomic soil. According to their observations nematode activity was dependent on solute composition and on water potential. In all solutions mortality ceased at a water potential of -30 x 10^5 Pa. A high level of activity was sustained in synthetic soil solution. Nematodes were killed at -15 and -16x 105 Pa in sodium and synthetic soil solution, respectively. Bilgrami *et al.* (1986) observed activity of *Hirschmanniella oryzae* in some chemicals. They found that low concentrations of sodium chloride, potassium chloride and ferric chloride (0.001 M) were favourable to the activity of *H. oryzae*. Ammonia solution suppressed activity at higher concentrations although it had least effect at 0.001 M. Similar observations were made by Khan and Khan (1990).who found that all the concentrations of sodium bicarbonate and sodium chloride (1.5, 2.5, 3.5 and 5%) significantly induced mortality of *Meliodogyne javanica* and *M. ingognita* juveniles. The extent of mortality was dependent on salt concentration which gradually increased with the exposure time at all salt levels. According to Gysels and Bracke (1975) a salt concentration of up to 15g NaCl / L in brackish water did not adversely effect survival and reproduction of *Panagrellus silusiae*; higher salt concentrations in sea water resulted in shrinkage of the nematode and lack of mobility.

2.2. Movement and Behaviour

Movement and locomotion activities of animals are biologically necessary for migration, obtaining food, shelter from enemies; escape from adverse environmental conditions and also to locate a potential mate (Azmi and Jairajpuri, 1975). According to Jairajpuri *et al.* (1974) as concentration of salts solution increases, nematode first exhibit the jerky movements before finally becoming inactive. In 0.4M mercuric iodide solution the nematodes die immediately forming irregular bends with their cuticle showing many folds. Al lower concentrations of salt solutions the worms show increased activity as if irritated on being introduced to the solution. The movements take the form of lashing, spiraling, and forming figures of '8'. The bending of the body is more often combined with side-ways rolling either of the whole or part of the body. This activity seems similar to the observed by Stephenson (1945) for *Rhabditis* and Banage and Visser (1965) for *Dorylaimus*. *X. basiri* and m. minor usually showed the movements of the posterior part of the body and only occasionally of the anterior part especially when approaching death. However, *Haplolaimus indicus* and *Helicotylenchus indicus* continued to show movements of the entire body even when very near to death.

Ibrahim and Hollis (1967) stated that *Tylenchiorhynchus martini* was attracted to 0.1M aluminium chloride and 0.25M calcium chloride but not at higher concentrations of these salts or to ammonium chloride, magnesium sulphate or sodium sulphate. Huettel and Jafri (1987) observed positive movement by *Heterodera glycines* to some ionic solutes and biological compounds. Males of *H. glycines* were highly attracted to potassium hydroxide and repelled by hydrochloric acid. Luedders *et al.* (1979) reported that the number of cysts of soybean cyst nematodes were increased when the soil was treated with potassium at 50, 100 or 200 mg/pot than in the untreated control whereas fewer cysts developed when the soil was treated with higher doses of potassium (400 mg/pot). The decrease in number of cysts in soil was larger with potassium chloride than potassium sulphate. According to Riddle and Bird (1985) the plant parasitic nematodes *Rotylenchus reniformis, Anguina agrostis* and *Maloidogyne javanica* were attracted to some chemicals: the least attractive salts were ammonium sulphate and magnesium sulphate whereas most attractive salt was magnesium chloride. Evans (1969) indicated that magnesium sulphate influenced the movement of males of *Heterodera rostochinensis*. The free-living nematode, *Caenorhabditis elegans* has been shown to be attracted to the anions Cl^-, Br^-, and I^-, and to the cations Na^+, Li^+, K^+, and Mg^{2+} (Ward, 1973). Prot (1978a) indicated that the juveniles of *M. javanica* placed in a salt gradient, the juveniles moved preferentially toward the region having the lower concentrations of a number of salts – sodium chloride, calcium chloride, potassium chloride, magnesium chloride, sodium nitrate, calcium nitrate, potassium nitrate, sodium dihydrogen phosphate, ferric chloride and magnesium sulphate. The repulsion was significant in created in agar by salt solution of concentrations between 0.125 x 10^{-2} M /L which is very close to the concentration found in soil. However, this repulsion was not observed in 2 x 10^{-2} M solution of calcium nitrate and calcium chloride where as with other salts e.g., sodium chloride and potassium chloride the repulsion was significant at this level of concentration. Ferric chloride did not significantly repel the nematode. In an another experiment Prot (1978b) described the tracks of *M.*

javanica juveniles movements in agar with salt gradient; tracks were generally straight, directional changes common only in a gradient created by a high salt concentration. It was concluded that all individuals have capacity to orientate toward a lower concentration in a salt gradient but because of random movement or some unknown factors, a very small percentage of individuals track in the opposite manner. When a concentration gradient nutritive salt solution was combined with a moisture gradient, the juveniles of *M. javanica* moved towards the region having the lower salt concentrations which also had the lowest moisture content (Prot, 1979a). This phenomenon was observed when a moisture gradient was combined with concentration gradient of potassium di hydrogen phosphate, calcium nitrate and magnesium chloride. In soils with an adequate amount of mineral salts, dilution of the soil solution along a moisture gradient could induce migration of juveniles to the region with lower salt concentration which is also the wet end of the moisture gradient. In another experiment, Prot (1979b) studied the influence of concentration gradient of salts (sodium chloride, potassium nitrate, calcium nitrate and magnesium sulphate on four plant parasitic nematodes. All three populations of *M. javanica*, and *M. incognita* migrated to the lowest concentrations of the four salts tested; juveniles of H*eterodera oryzae* were only repelled by sodium chloride but none of the salts repelled *Scutellonema cavenessisp*. It appears that the capacity to migrate towards the region having the lower salt concentrations is common with *Meliodogyne* species, however, it is not common for all plant parasitic nematodes.

3. Hatching of Nematode Eggs

The hatching of eggs is affected by several environmental factors including temperature, soil moisture, soil aeration, pH, and organic or inorganic chemicals in the soil water. The chemicals may be derived from the soil itself or from organic matter or soil inhabiting micro-organisms (Shepherd and Clarke, 1971).

Robinson and Heal (1956, 1959), Ellenby and Gilbert (1957, 1958) and Dropkin *et al.* (1958) have suggested that metal ions may be involved in the hatching mechanism of the potato cyst nematode, *Globodera rostochiensis*. Atkinson and Ballantyne (1979) revived the hypothesis that Ca^{2+} in particular has an active role in the initiation of hatching. Robinson and Neal (1956, 1959) suggested that a mixture of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} ions is essential for maximum activity of the hatching factor. According to Clarke and Hennessy (1983) *Globodera rostochiensis* eggs were stimulated to hatch by solutions of potato root exudates to which a range of 0.5 to 10mM of chlorides Na, K, Ca or Mg were added. They suggested that the initiation oh hatching may be the effect of changes in egg shell permeability brought by the effect of the hatching factor on bound Ca^{2+}. In tests, on *H. glycines*, Lehman (1969) found that the cations Mg^{2+}, NH_4^{+} were inhibitorier than Ca^{2+} and among anions NO_3^{-} was inhibitorier than Cl^{-} and SO_4^{2-}. Wallace (1956) compared the effects of some ions on *H. glycines*. He reported that Ca, Mg, Na, and K, all caused more larvae to emerge than water. Clarke and Shepherd (1966) tested the effects of many different ions on the hatching of nine *Hetrodera* species and confirmed that Ca, and Mg ions hatched *H. schachtii* a little but found that K and Na ions did not. The hatch of *H. rostochiensis* eggs was decreased when exposed to saline media (Everard, 1960). Thistlethwayte (1969) found that increasing concentrations of the Na and K salts decreased hatching of eggs of *Pratylenchus penetrans*. Similar trends were obtained in all the solutions but when the juveniles were transferred to distilled water hatching increased. Hypochlorite solution has been reported to stimulate hatching of eggs of some *Heterodera* species. Shepherd (1963) used calcium hypochlorite to hatch eggs of *H. goettingiana*. Fox and Kerkes (1969) used low concentrations of sodium hypochlorite to hatch eggs of *H. carotae, H. glycines* and *H. weissi*, whereas high concentrations killed larvae and also dissolved cyst walls, egg shells and cuticle.

The hatch of *M. javanica* and *H. rostochiensis* eggs decreased as the concentration of electrolytes (sodium chloride, calcium chloride and potassium chloride) increased in the media (Dropkin *et al.,* 1958). Similarly, hatching of *M. incognita* eggs decreased as salinity increased (Edongali and Farris, 1981). Lal and Yadav (1975) showed that hatching of eggs of *M. incognita* was inversely proportional to the concentrations of sodium and Calcium chlorides. They also noticed that when the eggs masses from different salinity levels were transferred to double distilled water the eggs revived their hatching ability; the salt concentration inhibited the hatching but did not kill the eggs of *M. incognita*. According to Yousif and Badra (1981) hatchability in *M. javanica* was reduced by several organic and inorganic compounds. They observed that hatching was prevented entirely by 10 and 5g urea and ammonium nitrate per liter while ammonium sulphate recorded 97 and 71% suppression at these two rates, respectively. There was a marked hatch in the lowest dilution of ammonium nitrate and ammonium sulphate and no suppression was caused by urea. According to Kanwar *et al*. (1989) when eggs of *M. javanica* were exposed to sodium hypochlorite at different concentrations, eggs showed less hatching at 750 and 1000 ppm than the control whereas the hatching was greater than the control at lower concentrations.

Maqbool *et al*. (1987) used sodium chloride and calcium chloride (0.03, 0.06 and 0.10 M) separately and in combination (0.03 NaCl + 0.03 $CaCl_2.2H_2O$, 0.06 NaCl + 0.06 $CaCl_2.2H_2O$, 0.1 NaCl + 0.1$CaCl_2.2H_2O$) with each other and found that hatching of *M. javanica* eggs decreased with increasing concentration. At all salt concentrations the number of emerged juveniles after 24, 48 and 72 hours were greater in sodium chloride treatment. Calcium chloride and Sodium chloride + Calcium chloride were more detrimental to the hatching of eggs. Juvenile infectivity was inversely proportional to the salt concentration. Up to 7 days juvenile penetration to tomato roots was relatively more in Sodium chloride than in calcium chloride or in combination of the two salts. Similar observations were made by Khan and Khan (1990) who tested various concentrations of sodium chloride and sodium bicarbonate and found that all the levels of salinity inhibited the hatching of *M. javanica* and *M. incognita* eggs and reduced their mobility. A negative correlation between hatching and salinity levels was recorded.

4. Role of Soil Salinity In Plant-Nematode Interactions

The role of salinity in plant-nematode interactions has been partially studied in Cotton (Heald and Heilman, 1971), salt tolerant tomato (Maggenti and Hardman, 1973), sweet orange (van Gundy and Martin, 1961), Citrus (Majrebi, personal communication) and okra and brinjal (Jain *et al.,* 1989).

i. HONEY DEW MELON: Nischwitz *et al.,* (2002) reported that in green house studies, higher concentrations of salts in irrigation water significantly increased the percentage of honey dew melon (*Cucumis melo* cv. Green Flesh) plants that died due to charcoal rot (*Macrophomina phaseolina* (Tassi) Gold, a soil borne microsclerotia producing fungus). Infection with root knot nematodes played no role in increased incidence of charcoal rot of melon – salinity was the alone factor. There was only 3% mortality in treatment with saline irrigation water of 0.53 $dS.m^{-1}$, 18% in 1.16 $dS.m^{-1}$, 37% in 3.10 $dS.m^{-1}$, 77 % in 6.23 $dS.m^{-1}$ and 94% in 11.55% $dS.m^{-1}$.

ii. COTTON: Heald and Heilman (1971) found that as the soil salinity increased the growth of cotton decreased nematodes significantly, reduced the fresh and dry top weights of inoculated plants. The stunting and lack of vigour appeared in all inoculated plants. As soil salinity increased the percentage in plant weight attributed to nematode injury increased. Dry weight of inoculated plants was reduced significantly in saline soil. Nematodes intended plants at high salinity levels dropped several bottom leaves and plants were very weak with thin stems. The reniform nematode attacks and damages cotton plants at all salinity levels, and nematode injury increases with soil salinity.

iii. OKRA AND BRINJAL: The plant growth of okra and brinjal decreased in saline soil inoculated with *M. Javanica*. At the higher salinity levels all okra and brinjal plants irrespective of nematode infection, were more stunted than at lower levels. With increase in salinity the reduction in growth was more pronounced in infested plants than in healthy ones. However, the root-knot index in both crops increased with salinity (Jain *et al.,* 1989).

iv. CITRUS: Salinity interacts with biotic pests and diseases including root rot (*Phytophthora* spp.), nematodes and mycorrhizae. Irrigation with high salinity water not only can have direct effects on root pathogens but it can also predispose citrus root stocks to attack by root rot and nematodes. Nematodes and mycorrhiza can affect the salt tolerance of citrus roots and may increase Chloride ion uptake. Not all effects of salinity are negative; as moderate salinity stress can reduce physiological activity and growth, allowing citrus seedlings to survive cold stress and can even enhance flowering after the salinity stress relieved (Sylvertsen and Levy (2005).

According to Majrebi (personal communication = Edongali and Ferris, 1980), with increasing salt concentration. *T. semipenetrans* populations on citrus were reduced as well as the weight of citrus seedlings. This may be attributed to a direct effect of salt on nematode and plant growth. Salt has been known to affect the nematode's chemotactic response towards its hosts, which in turn affects production and parasitism (Edongali and Ferris, 1980). Higher salt concentration may also lead to unavailability of other essential elements which results in reduction of plant growth.

The interaction among citrus rootstocks, salinity and *Tylenchulus semipenetrans* investigated by Mashela and Nthengeni (2002) indicated significant results ($p < 0.05$). Nematodes generally increased salinity ions in leaves and reduced ions (Cl, Na) in roots and K in both leaves and roots. Thus management of nematodes is critical in areas with salinity problems.

v. TOMATO: Tomato plants are rated as moderately sensitive to the presence of salt in the soil from the late seedling stage to maturity (Bernstein, 1959) Their growth and development are affected by ionic salts in the soil (Bernstein and Hayward, 1958). Yield reduction of about 10% occur at 3.5 $dS.m^{-1}$ and 30% at 5 $d.Sm^{-1}$ (Bernstein, 1964). According to Edongali and Ferris (1982) nematode infestation of salt stressed tomato plants which are nematode resistant (Beefmaster and Atkinson) and susceptible (cv Hunts 2580 and cv Ronita) significantly reduced plant height, fresh and dry weights, number of flowers, and fruit weight in most cultivars. In Hunts 2580, number of flowers and fruit weight increased. Nematode reproduction on susceptible varieties decreased with increase in salinity. The decrease in dry weight of tops and roots at 4 $dS.m^{-1}$ and increase at 8 $dS.m^{-1}$ may be due to the effect of salinity on absorption and plant tissue formation. The salt concentration at 4$dS.m^{-1}$ was not high enough to cause a notable increase in the osmotic pressure of the soil solution where as at 8 $dS.m^{-1}$ osmotic increased in the cell sap.. Thus although the plant height decreased at 8 $dS.m^{-1}$ the mineral absorption and cell sap concentration increased; this in turn would be reflected by an increased in dry weight of top and roots. According to El-Kholei *et al.* (1982) when the tomato cultivar Prichard was grown in *M. javanica* infested soil with salinity of 1000-9000 ppm sodium chloride, the dry weight of plants was decreased. **A lower level of nutrient in plants was found**.

Infectivity and Development of *Meliodogyne* Species on Tomato

According to Edongali *et. al.* (1982) infectivity of *M. incognita* on tomato varieties was lower on no-salt treatments of Cv. Beefmaster than cv. hunts. Beefmaster did not show strong resistance to nematode penetration. Calcium chloride treatment generally caused a significant reduction only at the higher salt concentrations. When the more susceptible cultivar hunts, was used increase in EC_e tended to reduce infectivity. Salts effects were generally more pronounced early in the experiment and each type of salts significantly reduced the infectivity. They also observed that the influence of salts on nematode development appeared more pronounced on Cv. Hunts than cv. Beefmaster. The development of mature females in cv. Beefmaster was delayed by salt treatments. The delayed development of the nematode resulted in fewer total number of nematodes and populations at the end of the season. Edongali and Ferris (1981) showed that the presence of salt resulted in higher infectivity on tomato by *Meloidogyne* species. Maximum infectivity was obtained by sodium chloride treated juveniles from all egg sources.

In an experiment (Maqbool *et al.,* 1989) whether NaCl or $CaCl_2$ were applied separately or in combination, the juvenile infectivity of *M. javanica* on tomato was found to be inversely proportion to the salt concentration. All three concentrations of NaCl had no significant influence on juvenile penetration (Contradictory statement). Up to 7 days in all salt concentrations, juvenile penetration and subsequent infectivity was more in NaCl followed by $CaCl_2$ and the combined salt treatment. In another experiment Maqbool *et al.,* 1990) studied the effects of these salts alone or in combination in cv. Roma VF, Roforto, Lyallpur selection, PARC I and PARC II. In vitro studies as concentration of either salt increased the number second stage (J2) penetrating roots of all cultivars decreased as compared to the non-treated plants. Number of J2 penetrating the roots, time of molting and duration of each molt was earlier in NaCl than in $CaCl_2$ and their combinations. Total number of third and fourth stage juveniles (J3 and J4) were significantly low but developed earlier and greater in all cultivars except PARC I regardless of salt type or salt concentration. In non-treated plants roots, large number of egg masses was produced by fourth week after inoculation, few in the low and none in the high concentration of $CaCl_2$ and combined treatment of the two salts. After five weeks few mature females with small egg masses were found. At the highest concentration (0.10M) of either salt or their combination the few J2s that penetrated roots were dead after 2 weeks within roots in all cultivars. The nematode developmental cycle was prolonged in all salt treatments in PARC I whereas calcium chloride only prolonged development in Cultivars Roma Vf, Roforto, PARC II and Lyallpur selection. In greenhouse studies Second stage juveniles penetration of roots and nematode development in all cultivars tested was affected by salt concentration. Root penetration by J2 and development of J2 / J4 was suppressed at high concentrations of both salts in all cultivars. The number of J3 / J4 was lower in all cultivars and mature females were fewer after three weeks of inoculation. In salt treated soil than non-treated soil. However, the development cycle was delayed in PARC I. The influence of salts on nematode development appeared to be more pronounced on tomato cv. PARC I than other cultivars tested. *M. javanica* reproduced an all cultivars, but Cultivars Roma VF, Roforto, Lyallpur selection and PARC II supported the reproduction regardless of the presence or absence of salts.

The effect of salt addition was more pronounced with calcium chloride and combined treatment than with sodium chloride alone. Four weeks after inoculation the maximum number of egg masses was observed in the non-treated control whereas in the high salt concentrations the number of egg masses was depressed. The calcium chloride treatment resulted in fewer females compared to the control or the NaCl. The combination of the two salts substantially suppressed the nematode development at all concentrations.

Salt types and concentration effects on nematode behaviour such as reduced penetration of J2 are in agreement with Edongali *et al.* (1982). There was, however, no indication that high salt concentration interfered with the nematode mobility. Juvenile penetration of roots was affected by all salt types and concentrations in all cultivars used in this study. The retarded root growth was observed in 0.06M of calcium chloride where nematode development was also retarded. At this concentration, NaCl had no effect on nematode development. At high concentrations these salts might have affected the developmental process of roots leading to unfavourable root tissues for nematode feeding (Mengel and Kirby, 1978). Since a healthy host plant is important for the development of its parasites (Tyler, 1933) nematode infection and developmental cycle might have been delayed at the moderate and high concentration of calcium chloride in these studies.

Nematodes in our agricultural systems have been the center of our interest for quite some years. The characteristics of nematofauna and its ecology in various natural ecological systems of Pakistan is, however, a neglected issue of research. Our diverse ecosystems - ice-laden areas in the North, arid plains and dunes, brooks, streams, rivers and their deltas and estuaries, Sea channels and mangroves, all are almost virgin areas of research in this respect. Essential research is needed on nematodes in natural and agricultural systems for disease suppression, for understanding and restoration of the environment and construction of better predictive models for land use decisions.

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