"INVESTIGATIONS ON YELLOWING OF BLACK PEPPER (*Piper nigrum* L.)"

By

MADDIRALA SURENDRA BABU

(2016-22-002)

THESIS

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA, THRISSUR - 680 656

2019

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

KERALA AGRICULTURAL UNIVERSITY

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2019

DECLARATION

I, **Maddirala Surendra Babu** (2016-22-002) hereby declare that, this thesis entitled **"Investigations on yellowing of black pepper (*Piper nigrum* L.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Place: Vellanikkara — Maddirala Surendra Babu

Date: — 2016-22-002

CERTIFICATE

Certified that this thesis entitled **"Investigations on yellowing of black pepper (*Piper nigrum* L.)"** is a record of research work done independently by **Mr. Maddirala Surendra Babu (2016-22-002)** under my guidance and supervision and that, it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the advisory committee of **Mr. Maddirala Surendra Babu (2016-22-002),** a candidate for the degree of **Doctor of Philosophy in Horticulture** with major field in Plantation Crops and Spices, agree that the thesis entitled **"Investigations on yellowing of black pepper (*Piper nigrum* L.)"** may be submitted by Mr. Maddirala Surendra Babu (2016-22-002), in partial fulfilment of the requirement for the degree.

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EXTERNAL EXAMINER

Dedicated To My Beloved Parents and
My Advisor Dr.V.S Sujatha

ACKNOWLEDGEMENT

I express by deepest sense of gratitude to 'Mother Nature' and the Almighty God for permitting me to reveal a little part of their handiwork, in the form of this research work.

I acknowledge with immense gratitude the input and encouragement of the people who helped me in this endeavour.

With immense pleasure, I avail this opportunity to express my deep sense of gratitude and indebtedness to my Major Advisor, **Dr. V. S. Sujatha** Professor and Head and Chairperson of my advisory committee, Department of plantation crops and spices, College of Horticulture, Vellanikkara, for her sustained and valuable guidance, constructive suggestions, unfailing patience, friendly approach, constant support and encouragement during the course of the research work and preparation of this thesis. She supported me during each step of this venture. I really consider it is my greatest fortune in having her guidance for my research work and will be remembered forever.

I express my heartfelt gratitude to **Dr. N. Mini Raj,** Professor, Department of plantation crops and spices, College of Horticulture and members of my Advisory Committee for their expert advice, valuable suggestions, support and cooperation throughout the course of study.

I am very much grateful to **Dr. P. Sureshkumar,** Professor and Head (Retd.), Radio Tracer Laboratory for the facilities provided, valuable guidance and wholehearted co-operation offered to me throughout the study. Without his utmost patience, keen interest and constructive suggestions, this venture would not have been completed successfully.

I extent my sincere gratitude to **Dr. Reshmy Vijayaraghavan**, Assistant Professor, Dept. of Plant pathology College of Horticulture, Vellanikkara for providing the necessary facilities to complete this study.

My deep felt thanks are due to **Dr. Berin Pathrose,** Assistant Professor, Dept. of Entomology, College of Horticulture, Vellanikkara for his valuable suggestions and the interest shown during the entire study.

I thank **Dr. Gavas Ragesh,** Assistant professor**,** Agriculture Entomology**.** Banana Research Station, Kannara for his constructive criticisms, suggestions and all the help rendered during the course of this investigation.

I am grateful to **Dr. S. Krishnan**, Department of Agricultural Statistics for the whole hearted cooperation and great help extended and great help extended for the statistical analysis of the voluminous data, in the interpretation of results and in the writing of the thesis.

I thank **Dr. Priyank Mahtrae,** ARS Scientist**,** Agriculture Nematology**.** ICAR-Central Potato Research Station, Ooty, **Dr. Uma Maheswari,** ARS Scientist**,** Agriculture Nematology**.** IIHR-Banglore for his suggestions and all the help rendered during the course of this investigation.

I wish to acknowledge **Dr. P. V. Nalini**, **Dr. K**. **Krishnakumary, Dr. Jalaja S Menon**, **Dr. B. Suma**, **Dr. Lissamma Joseph** and **Ms. Aneesha** in the Department of Plantation Crops and Spices for their support and encouragement during the course of work**.**

I render my profuse thanks to **Dr. C. Narayanankutty,** Associate Dean, College of Horticulture.

I am thankful to **Dr. Jayasree Sankar,** Professor and Head, **Dr. Beena** Assistant Professor, Soil Science and Agricultural chemistry and **Dr. Prameela**, Professor, Department of Agronomy, Professor and Head **Dr. AjithKumar** Agricultural Meteorology, Professor and Head **Dr. Anita Cherian** Department of Plant Pathology, College of Horticulture, Vellanikkara, for providing the necessary facilities and for their immense help and guidance, especially during the Pharmacology studies. I express my heartfelt gratitude to **Dr. Deepthi, Dr. Haseena**

Bhaskar, Dr. Smitha Department of Entomology for their valuable suggestions and support during my nematology study.

I thankful to **Dr. A. Sakeer Husian** Director to Center for E-Learning and In charge for Scholar hostel Warden for supporting and motivating during period of my research.

I am in dearth of words to express my sincere thanks to my classmate **Ms. Akoijam Ranjita Devi** for her affection and kind help offered not only in my research work, but also throughout my Ph.D programme.

I duly acknowledge **Mr**. **Danesh**, Farm officer, Department of Plantation crops and spices for providing all the help and support. I am grateful to **Mrs. Swapna K.M, Salomi, Raji, Lishi, Beena, Razhia, Radhika, Mr. Unni krishna and Mr. Sudheer**; **Mrs. Sheela** (Department of Agronomy) for their immense help and cooperation during research work. I also thank the non-teaching staff of Department of Plantation crops and Spices, **Sumi chechi**, **Sindhu chechi** and **Aishwarya chechi** for their cooperation.

I owe my thanks to my beloved seniors **Mr. Vijayakumar B. N., Ms. Nimisha Mathews,** I thank all my dear juniors **Mrs. Surya Raj, Mrs. Sruthy K., Ms. Geethumol Thankappan, Ms. Manisha Elza Jacob** and **Ms. Sreelekshmi S., Ms. Anila Peter** and **Ms. Dharini Chittaragi** whose support, help and encouragement throughout the Ph.D programme could never be forgotten. I owe thanks to **Mr. Manohar Lal Meghawal, Sarvana Kumar, Lalit Dhurvae, Sunny Babu, Arjun, Shobha Pujari, Lokesh, Silpa, Jeevan, Dr. Ambavane Ajinkya and Dr. Rajakumar (Soil Science)**. I thank all my dear juniors **Ms. Sarga, Ms. Anu, Ms. Megha** and **Mr. Sankar**. I thank all my dear PhD juniors **Ms. Aparna, Ms. Surya Subin, Ms. Suchitra.** I am also thanking to all juniors from Andhra pradesh for their help and encouragement.

I am also grateful to all farmers **Bose, Ameena, Joseph** and **Dr. Anitha** from Thrissur and who were the helpful for my survey and during my research work.

I owe special thanks to **Library, College of Horticulture** and **Kerala Agricultural University Central Library.** I wish to express my thanks to **Kerala Agricultural University** for the financial assistance offered during the study period.

I reveal my extreme sense of regards to my father, **Mr. Venkata Subbarao**; my mother, **Mrs. Nagendramma**; My grandmother **Mrs. Annapurnamma**, my brother **Mr. M. Ramanjaneyulu** and my sister in law **Mrs. Swathi**, My niece **Bhanu, Servani**, My mama **Mr**. **Ragavaiaha** My sister **Mrs. Kavitha**, My brother in law **Mr. M. Srinivasa Rao, my niece Anusha, Deepika** for their sacrifices, moral support, love, concern, prayers and blessings.

A word of apology to those I have not mentioned in person and a note of thanks to one and all who worked for the successful completion of this endeavour.

I render my profuse thanks to our teachers and spiritual parents **Dr. P. Ashok, Dr. Sasikala, Dr.N. Emmanuel, Dr. Kiran Pathro**, our beloved brothers **Dr. M. M. Nagaraju, Dr. T. Thomson, Dr. G. Koteswara Rao, Dr. Ravi Kiran,** My degree friend **K.Visweswara Reddy,** Our childhood friends **G. Venkateswara Rao, Ravi Anna, Nagababu and Ramesh, Varaprasad** I thankful for moral support, love, concern, prayers and blessings, College of Horticulture, Vellanikkara, Tadepalligudem Andhra Pradesh.

Dated:

Place: **M. Surendra Babu**

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Introduction

1. INTRODUCTION

Black pepper popularly known as “King of Spices” or “black gold” is one of the important and earliest known spices produced consumed large quantity domestically and exported from India. Kerala and Karnataka account for a major portion of production of black pepper in the country.

India, has an area of 1,35,915 ha under black pepper with production of 64,000 tonnes and productivity of 471 kg/ha (Spices Board, 2018). Black pepper production is showing shrinking trend in the recent years. Production and productivity of black pepper have suffered a severe setback during the past several decades due to improper management and rampant incidence of pests and diseases. After foot rot disease, the major constraint in black pepper cultivation is the incidence of yellowing where the production is found to decline year after year.

Yellowing in black pepper is a slowly debilitating disease reported to be caused by many factors like poor soil health, improper management practices and changes in climatic factors leading to biotic and abiotic stresses. Among the abiotic factors, stress imparted due to inadequate soil moisture, water stagnation, deficiency of nutrients, scorching due to over exposure to sunlight are reported to be associated with this pernicious problem, while diseases incited by a myriad of pathogenic microbes such as fungi, virus, nematodes and sap feeding insects are placed under the category of biotic factors for yellowing.

Among nutrients, deficiency of nitrogen, potassium, calcium, magnesium, iron, manganese, boron *etc.* are reported to cause chlorosis, yellowing, and retardation of growth and yield in black pepper.

Major plant pathogenic organisms involved in yellowing, growth retardation and death of plants are *Fusarium*, *Phytophthora* and *Rhizoctonia.* Pests like root mealy bugs and scales are also reported to cause yellowing in black pepper.

Plant parasitic nematodes either alone or in association with fungi resulted in yellowing and ‘slow decline’ of black pepper in most of the black pepper growing

countries. The major nematodes involved are root knot nematode *Meloidogyne* spp. and burrowing nematode *Radopholus similis*.

In addition to these biotic factors and soil and plant nutrients, environmental factors like low soil moisture and high temperature were also reported to induce yellowing in black pepper.

Thus it can be assumed that yellowing in black pepper is a symptom which is a manifestation of different causes either biotic or abiotic, either alone or in combination with other factors. Yellowing of pepper vines at the fag end of monsoon season was reported as a severe problem in Wayanad district, which is a pepper belt of Kerala. Extensive acidification and excess levels of phosphorus fertilization had lead to wide spread deficiencies of potassium, calcium, magnesium and boron which were the major limitations to crop production (Sreekumar *et al.* 2014)

Wahid and Kamalam (1982) reported that foliar yellowing and necrosis of distal ends of lamina in slow wilt affected gardens were due to N and K deficiencies. The disappearance of these symptoms with the onset of monsoon was attributed to increased nutrient uptake and their reappearance after the monsoon season to soil moisture stress and a consequent reduction in nutrient uptake.

Recently, there were reports on development and spreading of yellowing, as a major problem in cultivation of black pepper in Thrissur district of Kerala. To understand the problem and cause of yellowing, the present study was formulated with the objectives to find out the role of soil nutrients, plant pathogens, nematodes and mealy bugs in causing yellowing of black pepper and to develop suitable management strategies for controlling the yellowing.

Review of literature

2. REVIEW OF LITERATURE

Black pepper is affected by several diseases caused by fungi, bacteria, virus and mycoplasma besides insect pests, nematodes and nutritional disorders. Crop loss due to diseases and pests were identified as major causes of low productivity of pepper in India (Sarma and Anandraj, 1997).

Yellowing was reported as a serious problem in black pepper cultivation. Several biotic and abiotic factors have been attributed to yellowing in black pepper. Among abiotic factors, soil and plant nutrients and weather parameters were reported to have a role in yellowing. Among biotic factors, soil micro-organisms like *Phytophthora*, *Fusarium*, nematodes and soil borne insects like root mealy bugs were reported to cause yellowing in black pepper.

In this chapter the role of various abiotic and biotic factors causing yellowing in black pepper is reviewed in detail.

2.1 RHIZOSPHERE SOIL CHARACTERS AND THEIR ASSOCIATION WITH YELLOWING

2.1.1 Aluminium

De Waard and Sutton (1960) reported that aluminium toxicity caused green to yellowish discoloration, drooping of leaves with initial symptoms of necrotic spots with yellow halo along the main veins. However, liming the soils alleviated this toxicity.

Veloso *et al*. (1995) reported that black pepper (*Piper nigrum* L.) was usually grown in soils of low natural fertility and high aluminum saturation. The initial symptom of Al toxicity was a slower development and thicker roots, when aluminum supply increased from 0 to 15 ppm, there was a higher uptake of P, K, Ca, Mg, Mn, Fe and higher rates caused nutritional disturbances and reduction in growth.

2.1.2 pH

Martini *et al*. (1977) suggested lime to increase soil pH from 4.8 to 5.7 and to reduce exchangeable Al to 1.5 meq 100^{-1} g soil as a more effective means of optimizing the yield than rising of soil pH to neutrality.

Maria *et al. (*1985) found that liming raised the pH values insignificantly. Samonte (1985) obtained optimum yields when the pH was raised above 6. The nitrogen status of plants was improved by lime application.

Several workers have reported that application of lime decreased aluminium saturation and increased pH and exchangeable calcium content of soil (Lin *et al*., 1988 and Broadbent *et al.*, 1989). Nakayama *et al*. (1987) found that liming increased nitrogen, phosphorus, potassium, calcium and magnesium contents of the soil.

Sreekumar 2015 reported that in yellowing affected areas in Wayanad pH was 3.5 which was ultra-acid. 55% of the samples fell between pH values 4.5-5.5 extremely acid to strong acid.

2.1.3 Soil nutrients

Mc Lean (1970) noticed that liming had little favorable effect on phosphate availability to plants in highly weathered semitropical and tropical soils because of the presence of so much reactive surface area composed of Al and Fe hydroxides or hydroxy-Al-hydroxy-Fe ions for fixing P.

The amelioration of subsoil acidity through surface application of amendments dependent on transport of base cations from the surface horizon and the reaction of these cations with the acidity in the subsoil horizons. Transport was dependent on the amount of water and the concentration of cations in the leaching water, latter was dependent on concentrations of accompanying anions such as sulfate (SO_4^{2-}), nitrate (NO_3^-), chloride (Cl^-) and bicarbonates (HCO_3^-). The reaction of subsoil acidity dependent upon the ability of the base cations in solution to displace

or react with the exchangeable Al^{3+} on subsoil particle surfaces, determined by the ratio of acidity to base cations in the incoming leachate (Pleysier and Juo, 1981; Pavan *et al*., 1984 and Cahn *et al*., 1993).

The role of lime materials (burnt lime or quick lime, slaked lime, calcite, dolomite and limestone) in reducing solubility of Al, Fe, Mn *etc*. and increasing nutrients availability of Ca & P and crop yields have been reported by Mandal *et al*. (1975) and Tripathi *et al.* (1997).

Nambiar *et al*. (1965) worked out tentative ratios of K_2O (total)/N, K_2O (available)/N and $CaO + K_2O + MgO$ /N in soil and found that slow decline of pepper occurred when these ratios were below 14.1, 0.05 and 3.8 respectively. The adoption of integrated nutrient management together with disease and pest management brought down the incidence of *Phytophthora* foot rot from 6.1 to 1.9 per cent and slow decline from 6.4 to 2.2 per cent.

De Waard (1979) reported that application of appropriate dose of fertilizer with extra amount of K and other nutrients like Ca and Mg in combination with mulch was found effective in controlling yellow disease of black pepper.

The deficiency of Zn, Fe and B and the lack of equilibrium between P and K and relation to Ca and Mg predisposed the black pepper vines to *Fusarium* wilt infection in Brazil. (Duarte and Albuquerque, 1991).

Hamza *et al*. (2004) reported that influence of soil characteristics of major black pepper growing soils recorded high pH. The yield was low due to low amount of organic carbon, phosphorus, calcium, magnesium and zinc. Sadanandan *et al*. (2002) reported an increase in almost all the nutrients and yield due to the adoption of integrated nutrient management involving recycling of FYM.

Hamza *et al.* (2007) reported that the soils of high yielding black pepper gardens were sandy to loam textured with near neutral pH, high in exchangeable bases, organic carbon and micronutrients especially zinc.

Hamza *et al*. (2007) revealed that the soils in most of the black pepper gardens were acidic (pH 4.4 to 6.7) in Kerala and Karnataka. Most of the soil samples analysed showed that OC, Zn, P, Ca and Mg level were low and plant samples also have recorded low Mg, Cu, P, K, Zn and Mg.

Wayanad is a major pepper growing tract in Kerala. During the early part 21 st century pepper gardens in Wayanad were showing symptoms of yellowing, which was spreading to adjacent plants and in severe conditions the whole plant turned lemon yellow in colour. There was severe reduction in yield, and the problem was known as post monsoon yellowing. Yellowing of pepper vines at the fag end of monsoon season was a serious problem in Waynad district. There was no new growth, affected leaves showed marginal yellowing and interveinal chlorosis, which turned into general yellowing later and was different from post monsoon yellowing. It was a complex issue caused by severe deficiency of magnesium and potassium. It was reclaimed by INM, IPM practices which were followed for two years. Application of gypsum was beneficial and reduced severity of the yellowing in the initial days (Sreekumar, 2015).

Sreekumar (2015) reported that extensive acidification; excess levels of phosphorus and widespread deficiencies of potassium, calcium, magnesium and boron were the major limitations to crop production in the pepper growing tracts of Wayanad. Organic carbon was low in 21 per cent samples, only 21 per cent had low P, 38 per cent had medium P and 41 per cent samples had high P. Heavy input of phosphatic fertilizers lead to build up of very high levels of nutrients in soils, which adversely affected the absorption of micronutrients like zinc and boron by pepper. 62 per cent of the samples were deficient in potash, 70 per cent of the samples showed

deficiency in calcium, 83 per cent showed adequate level of sulphur and 58 per cent of the samples were deficient in zinc.

Aloka (2016) reported that gypsum as an amendment either alone or in combination with burnt lime and dolomite reduced the soil and subsoil acidity and increased the available nutrient status in the surface as well as subsurface soil layers, which might have resulted in better proliferation favouring vigorous plant growth and development in acid soils. The results indicated that low pH directly inhibited root development and function, limited the K, Ca and Mg absorption and reduced seedling growth. At pH 5.5, black pepper attained maximum growth, while the minimum growth occurred at pH 3.5. It was concluded that low pH reduced plant growth and was associated with low root nutrient concentrations of K, Ca and Mg.

2.2 NUTRIENT DEFICIENCY AND YELLOWING

De Waard (1969) reported a critical level of two per cent K in the plant as a limit for K deficiency. Azmil and Yau (1993) reported that the nutrient removal by pepper plants was N - 255, P - 22.8, K - 208.2, Ca - 54.5 and Mg - 36.4 kg ha^{-1}. Riga and Anza (2003) reported that under Mg-deficiency, black pepper plants showed a decrease in relative growth rate, total dry weight and total leaf area.

Foliar yellowing and necrosis of the distal ends of laminae, in diseased vines, were attributed to N and K deficiencies, respectively. The disappearance of these symptoms with the onset of the monsoon was attributed to increased nutrient uptake, and their re-appearance, after the monsoon season, to soil moisture stress and a consequent reduction in nutrient uptake. In pot trials, despite a high soil organic matter content, the addition of N was necessary to prevent foliar yellowing (Wahid and Kamalam, 1982).

Wahid and Kamalam (1982) reported that foliar yellowing and necrosis of distal ends of lamina in slow wilt affected gardens were due to N and K deficiencies

respectively. Moreover, K levels of the healthy vines were considerably higher than those of diseased ones and concluded that K deficiency is one of the causes for slow decline of pepper vines.

Wahid and Kamalam (1982) observed that foliar yellowing of leaf samples was due to N deficiency and it got disappeared after the onset of monsoon due to increased nutrient uptake.

Sushama *et al*. (1984) concluded that the first mature leaf of fruiting laterals just before flushing was most suitable for foliar diagnosis. Leaf analysis results showed that 46 per cent samples had Mg, 39 per cent samples had Cu and 12 per cent samples had P, K and Zn status below the critical values. The order of limiting nutrients was: OC > Zn > P >Ca> K > Mg for soil and Mg > Cu > P=K=Zn >Mn for leaf samples.

Nybe and Nair (1987) reported that deficiency of calcium can cause yellowing in black pepper. Symptoms were observed under sand culture experiments. Due to the deficiency of calcium, symptoms appeared as tiny brown necrotic pinhead spots over chlorotic area near the leaf margins (initial stages). Symptoms were first observed on immature leaves followed by mature ones. The chlorotic area spread towards the distal end of the leaf (medium stage).

Nybe and Nair (1987) reported that sulphur deficiency of black pepper plant showed symptoms that first manifested on the younger leaves which turned uniformly yellow. Moreover, growth of vine was completely arrested at a very early stage.

2.2.1 Nitrogen deficiency

Nitrogen deficiency symptoms were generally characterised by poor growth, with pale and yellowish leaves. At first, the lower leaves turned yellowish. But the upper canopy of affected plants remained relatively green. In severe cases, leaves of the entire plant showed a characteristic yellow to orange yellow discolouration and

the extreme end of the leaf tip became necrotic in some instances. Leaf abscission was common in severely affected plants (George *et al.*, 2005, similar results were reported by De Ward, (19690, Nybe and Nair, (1987), Wahid and Kamalam (1982).

2.2.2 Phosphorus deficiency

Clear symptoms of phosphorous deficiency were often rare in the field. In severe cases, the most striking symptom was stunted growth of the plants. This affects more of restricted lateral growth due to poor secondary branching. Leaf blades of mature leaves became very dull looking, turned bronze coloured, was stiff and showed necrosis at the tips in some instances, before abscission occurred (George *et al.*, 2005). Similar results were reported by De Ward, (1969) and Nybe and Nair, (1987).

2.2.3 Potassium deficiency

In potassium deficiency distal end of affected mature leaf blades became necrotic, brittle and grey in colour. Necrosis was usually confined to the distal end, while the portion beyond the boundary separating necrotic and live tissues displayed a 'V' shaped band, which was yellow to reddish brown. This band sometimes occurred without the 'tip burn' symptom (George *et al.* 2005). Similar results were reported by De Ward, (1969) and Nybe and Nair, (1987).

2.2.4 Calcium deficiency

Visually, calcium deficiency was first observed in fresh mature leaves as yellowing or chlorosis which started on either or both edges near to the petiole end or middle part of the leaf blades. The marginal chlorosis advanced inward, followed by necrosis. The proximal and distal ends of the affected leaves were either green or pale green. Tiny pinhead necrotic spots were scattered between the main veins on the lower and upper surfaces of the leaves. Leaf abscission occurred before the central portion of the leaf turned necrotic. Die- back occurred at the growing point. Leaves of

the lower canopy were usually more severely affected than those of upper canopy (George *et al.* 2005). Similar results were reported by De Ward, (1969) and Nybe and Nair, (1987).

2.2.5 Magnesium deficiency

Magnesium deficiency symptoms first appeared on older leaves and progressed to younger leaves. In the early stage, chlorosis occurred in between main veins. This usually started from the central proximal half of the leaf. The chlorotic area enlarged to the leaf tip and subsequently towards the leaf margin. A light dramatic leaf fall was often induced, leaving the branches quite bear with only younger unaffected leaves remaining on the plant. The area near the petiole often stays green and gives an arrowhead effect of green tissue penetrating the yellowing areas on the leaf (George *et al.* 2005). Similar results were reported by De Ward, (1969) and Nybe and Nair, (1987).

2.2.6 Iron deficiency

Iron deficiency started in the younger branches and was characterized by interveinal chlorosis. The chlorosis occured in between the main veins and smaller veins as well, forming a fine reticulate pattern of green veins contrasting sharply with a pale green or yellow background. The youngest leaves were turned completely green or even white. In acute deficiency, the internodal length of terminal shoots and lateral branches were markedly shortened and leaves crowded together at the upper end of the canopy. The berries of affected vines appeared pale green to yellow (George *et al.*, 2005). Similar results were reported by De Ward, (1969) and Nybe and Nair, (1987).

2.2.7 Manganese deficiency

Manganese deficiency symptoms were more severe in the upper canopy of affected plants. Younger leaves turned chlorotic or yellowish white with only the

main veins remaining green. The older leaves showed a characteristic herringbone pattern with green veins and the areas between the veins turned yellowish white. At a later stage, small necrotic spots appeared and enlarged in the pale areas. Manganese and iron deficiencies occurred simultaneously as both were induced by over liming. While the symptoms of young leaves in a manganese-deficient plant could easily be confused with those of iron, the two deficiency symptoms were distinctly different in the mature leaves. When deficiencies were prolonged and severe, berries also showed characteristic symptoms (George *et al.*, 2005). Similar results were reported by De Ward, (1969) and Nybe and Nair, (1987).

2.2.8 Boron deficiency

Boron deficient plants were stunted with shortened internodes and reduced branching. Young and recently matured leaves showed characteristic symptoms of interveinal chlorosis at the distal and central portion. Young leaves were small and distorted with pronounced puckering and necrotic lesions on the main veins (George *et al.*, 2005).

2.2.9 Acid soil conditions

Necrotic spots developed along the main veins and between the main veins in the middle portion of the affected leaves. More than half of the distal portion of mature leaves became chlorotic. Symptoms in recently mature leaves were more severe than those of the younger and older leaves. In immature pepper, growth was severely retarded. The root system of affected plants developed poorly, having black and decayed roots, which were brittle. Leaves shed prematurely and the yield of affected vines was poor. These symptoms were similar to those that were described as 'Aluminium' toxicity and multiple deficiencies (George *et al.*, 2005).

2.2.10 Manganese toxicity

Young leaves were normal while older leaves were affected. Dark brown to black spots appeared first at the leaf margin before extending towards the central portion of the lamina. This eventually led to a striking interveinal pigmentation. Premature shedding of leaves and poor secondary branching were observed in cases of acute toxicity (George *et al.*, 2005).

2.2.11 Aluminium toxicity

Surface applied gypsum ($CaSO_4$. $2H_2O$) efficiently and sustainably reduced Al saturation in naturally acidic subsoil through the incorporation of Ca^{2+} and exchange of Al^{3+} in the subsoil without neutralizing the subsoil acidity (Wendell and Ritchey, 1996 and Toma *et al*., 1999).

Pavar and Marshall (1984) considered exchangeable Al as the major criterion of soil acidity rather than hydrogen ion concentration, due to which Al toxicity causes poor root penetration, as well as reduced plant growth.

Higher concentrations of aluminium caused severe obsession of shoot and root growth. Older plants showed higher Al tolerance than young ones. There was a relationship between sensitivity of the plants to Al and ability to increase solution pH. With the increase in the concentration of aluminium, the concentration of phosphorus, calcium and iron decreased to $1/10^{th}$ of the original (Aniol, 1996).

Hutchinson (1983) observed that aluminium ions were potentially toxic to plant roots. Soil acidity was a major constraint for crop production worldwide, and yield losses were frequently attributed to aluminium toxicity (Foy, 1983).

2.3 STUDY ON ASSOCIATION OF PLANT PATHOGENIC ORGANISMS WITH YELLOWING

2.3.1 Phytophthora

Phytophthora foot rot caused by *Phytophthora capsici* was the most destructive disease in black pepper (Alizadeh and Tsao, 1985) followed by slow decline mainly caused by the association of burrowing nematode *Radopholus similis* and root knot nematode *Meloidogyne incognita* with *P. capsici* (Anandaraj *et al*., 1996; Ramana and Eapen, 1997). Plant parts like leaf, laterals, stem, spike and roots were susceptible to infection by *Phytophthora* sp. Slow decline was another important debilitating disease. The affected vines showed various levels of feeder root damage followed by foliar yellowing.

There were at least 3 destructive diseases of *Piper nigrum* in Indonesia, namely foot rot, yellows and stunted growth diseases, caused by pathogens acting singly or in combination. Foot rot disease caused by *Phytophthora capsici*, had become a prime constraint for *P. nigrum* cultivation in Indonesia, followed by yellows disease caused by a complex of causal agents including parasitic nematodes (*Radopholus similis* and *Meloidogyne incognita*) and stunted growth (which was suspected to be caused by a mycoplasma-like organism (MLO) (Sitepu and Kasim, 1991).

Anandaraj *et al.* (1994) conducted experiments under simulated field conditions to investigate the effects of plant age on the resistance of *Piper nigrum var*. Panniyur-1 to different degree of artificial inoculum with *P. capsici*. Symptoms of gradual decline were recorded over a two year period, including yellowing, wilting and drying up of some branches and defoliation.

Jose Abraham *et al*. (1996) developed a simple method for indexing *Phytophthora* and nematode infection in black pepper.

Foot rot was one of most destructive disease of black pepper in Sarawak, which affected the total production of black pepper in Malaysia (Ravindran, 2000).

Phytophthora foot rot caused by *P. capcisi* was reported as a major devastating disease of black pepper plants causing a crop loss of 25-30 per cent in Kerala and 44-48 per cent of vines in Karnataka (Mammootty *et al.* 2008). Crop loss around 1000 tonnes annually was reported in Kozhikode and Kannur districts in Kerala due to foot rot disease (Devasahayam *et al*., 2008).

MDSiti *et al*., 2013 reported the causal agent for foot rot disease in black pepper in Sarawak as *Phytophthora capsici.* Out of 13 major pepper areas, Ulu sarikei had highest disease incidence and disease severity (75%, 70%) and the lowest (5 %, 4%) incidence and severity respectively was at Tatau. The affected plants showed yellowing leaf symptoms on both the upper and lower leaf canopies in all the sites studied.

2.3.2 Phytoplasma

Black pepper (*Piper nigrum*) yellows was reported from Coorg (Kodagu) district of Karnataka, India. Symptoms included yellowing and curling of the leaves. In the advanced stage, vines became yellow and slender, and a generalized decline in yield was observed. Based on observation of symptoms, an association of a phytoplasma with the disease was suspected (Adkar-Purushothama *et al*., 2009)

2.3.3 Fusarium

The black pepper vines remained healthy until large portions of the roots were damaged. In the advanced stages of the root rot, plant showed foliar yellowing, and shedding of leaves, spikes and lateral branches were noticed. The amount of defoliation due to root rot infection was equal to root damage. The root loss to regeneration determined the spread of the decline and death of the vine. During the post monsoon season with depletion of soil moisture, the remaining root system was

unable to support the vine, so that the entire vine collapsed with wilting and drying of leaves. Foliar yellowing, flaccidity, defoliation, breaking of the stems at nodal regions and spike shedding were the characteristic aerial symptoms of root rot and collar rot infections (Muller, 1936; Holliday and Mowat, 1963).

Albuquerque (1961) identified the causal agent of yellowing disease as *Fusarium solani* f. sp. *piperis*. The most serious diseases of black pepper were ones caused by fungal pathogens and plant parasitic nematodes (Bong and Saad, 1986). Fletcher (1994) reported *Fusarium solani* was a common soil-borne fungus and a pathogen of many agricultural crops such as pepper

Sitepu and Mustika (2000) reported crop loss due to *Phytopthora* rot was 10 - 15 per cent in Indonesia.

Koike *et al*. (2000) in a study on genetic characterization of *F. solani* and to clarify phylogenic relationships, as well as useful for efficient management of yellowing disease and finding breeding programmes to find cultivars of black pepper that were resistant to *F. solani* reported that disease management was difficult due to the presence of several pathogen types.

Fusarium infection in black pepper plantation has been reported to reduce the economic life of the plantation from 20 to 6-8 years and the productivity per plant from 3.0 to 1.5 kg (Anandaraj, 2000).

The symptoms of *F. solani* infection in the glass house-grown plants were yellowing of the foliage and root rot, leading to flaccidity. All black pepper plants inoculated with *F. solani* displayed yellowing symptoms four months after inoculation. At the end of the experiment (seven months after inoculation), of the 48 plants inoculated with *F. solani*, only four (8.3 per cent) plants displayed flaccidity, and these plants died within one week after flaccidity (Shahnazi *et al*., 2012).

Sreekumar (2015), reported a slight incidence of *Fusarium* spp. in almost all yellowing affected gardens in Wayanad.

2.4 ANALYSIS OF RHIZOSPHERE SOIL AND ROOT SAMPLES FOR NEMATODES

Several plant parasitic nematodes belonging to different groups were reported in association with pepper. Based on their parasitic habits they could be classified as ectoparsites, endoparasites and semi-endoparasites. Further, they were grouped as migratory or sedentary based on their movement in host plant tissues. The composition of plant parasitic nematodes associated with pepper was reported in the detailed surveys conducted during 1980s in Kerala and two districts in Karnataka (Ramana and Mohandas 1987). A new species as a semi-endoparasitic nematode, *Tropotylenchulus piperis*, was reported on pepper from India (Mohandas *et al*., 1985). The occurrence of this nematode on pepper has not been reported from any other country. In Indonesia, 14 genera of the plant parasitic nematodes were associated with pepper (Mustika and Zainuddin 1978, Bridge, 1978). Among them *Meloidogyne* spp., and *Radopholus* were predominant (Mustika, 1990). According to Sher *et al*. (1969), *Meloidogyne sp*., *Tylenchuluss semipenetrans* and *R. reniformis* were more prevalent in black pepper plantations in Thailand. In para, Brazil, *M. incognita, Xipnema sp.,* and *Helicotylenchus sp*. were commonly associated with black pepper. (Freire and Monteiro, 1978). In Srilanka, root knot and burrowing nematodes were of common occurrence in pepper. (Lamberti *et al*., 1983). The economic damages caused by many of these species were yet to be established. However, *Meloidogyne sp*., and *R. similis* were of much economic significance as they caused severe damage to pepper and were implicated in the slow decline/ yellow disease, a major production constraint in all pepper growing countries. Though *T. piperis* was very much prevalent with high infestation levels, its impact on pepper cultivation was yet to be evaluated and research in this direction is in progress in India.

Rashid *et al*. (2017) conducted a random survey in black pepper (*Piper nigrum* L) gardens of Wayanad district in Kerala state during 2015-16 and composite soil samples were collected from the root zone of black pepper. Several plant parasitic nematodes have been encountered from the rhizosphere of black pepper. Among them, *Hirschmanniella* sp. was found from four different locations of Mananthavadi taluk of Waynad district, Kerala. An average of 19 nematodes occurred in 100 cc soil. *Hirschmanniella sp*. was identified with the help of morphological characterization. Effects of soil pH, soil type and organic content (%) on *Hirschmanniella* sp. population were also assessed. Maximum *Hirschmanniella* sp. were recovered from sandy (50 per cent), followed by sandy loam and clay loam soils (25%, respectively). *Hirschmanniella* sp. occurred more in acidic soil (75%). *Hirschmanniella* sp. was reported for the first time from black pepper rhizosphere. This nematode was feared emerged as serious threat to black pepper cultivation due to endoparasitic habits.

2.4.1 Root Knot Nematodes (Meloidogyne sp.)

The first record of root–knot nematode infestation on pepper was reported from Cochin – China presently a part of Vietnam by Delacroix (1902). Almost during the same period, Ridley (1912) observed root-knot nematode infestation on pepper in Waynad, Kerala, India. He described a series of tumuors (root knots) on plant tissue due to the eel worm (*Heterodera radicicola = Meloidogyne incognita*) and that when these tumuors decayed it was not easy to detect the remains of the eel worms.

Ayyar (1926) reported the widespread occurrence of root knot nematodes in Wayanad. Root knot nematode infestation were also reported from many pepper growing countries like Malaysia (Holliday and Mowat 1963; Kueh, 1975; Ting, 1975; Razak, 1981), Indonesia (Ichinohe, 1976, Bridge, 1978), Brazil (Sharma and Loof 1974; Ichinohe, 1975); Thailand (Sher *et al*., 1969), Fiji (Swaine 1971), Guayana (Biessar, 1969) and Srilanka (Lamberti *et al*. 1983).

Among the four major species of Meloidogyne, *M. incognita* was reported as a major parasite on pepper. Three species namely, *M. incognita*, *M. javanica* and *M. arenaria* were reported on pepper in Sarawak (Kueh, 1975), the first two were widley distributed (Kueh and Sim, 1992). However, Siti Hajijah (1993) found that only *M. incognita* was widley distributed in all plantations surveyed in Sarawak and both healthy as well as diseased plants (pepper plants showing foliar yellowing) harbored the nematode. In Srilanka, *M. arenaria* was also observed to affect the growth of black pepper (Lamberti *et al.,* 1983). In Kerala and Karnataka about 70 per cent and 54 per cent plants, respectively were found infested with *Meloidogyne incognita* and both apparently healthy and slow decline affected vines harboured high populations of the nematode (Ramana and Mohandas, 1987).

Root knot nematodes were reported as sedentary obligate endoparasites. They have specialized and complex relationship with host plants. Infestation by them lead to the development of elongated swellings on thick primary roots due to multiple infections and typical knots or galls on secondary/ fibrous roots due to hypertrophy and hyperplasia of the infested tissues. In thick roots several adult females with egg masses were situated deep below the epidermis and the whole length of the root turned in to a gall and hence appeared almost smooth with occasional swellings here and there (Mohandas and Ramana 1987).

Certain physiological changes were also observed in plants infested with *M. incognita,* like reduction in absorption and translocation of P, K, Zn, Mn, Cu, Ca and Mg and these elements accumulated in the leaves (Ferraz *et al*., 1988).

Pepper plants infested with root knot nematodes generally exhibited foliar yellowing, poor growth and gradual decline in health and vigour. Sometimes leaves of infested vines showed dense yellowing of inter veinal areas making the leaf veins quite prominent with deep green colour (Ramana, 1994). Kueh (1979 and 1990) reported that in the plants infested with root knot nematodes, leaves were held inward

and upward followed by defoliation. In the pathogensity trials with *M. incognita* and *Fusarium solani,* Mustika, (1990) could not reproduce the symptoms like stiff droop and yellowing of leaves in plants inoculated with *M. incognita* alone. Similarly, severe foliar yellowing could not be observed in the plants inoculated with lower doses of nematode inoculums in pathogensity tests conducted in India under simulated field condition (Mohandas and Ramana 1991). Nematodes occupied the stellar portion of roots and fed on giant cells. In due course many giant cells coalesced and stellar portion was completely destroyed (Mustika 1990).

Since 1992 black pepper vines in Brazil, cultivar Guajarina growing in the field for more than four years had been affected by yellow wilt. The pathogen invaded pepper through wounded roots inflicted by nematodes *M.incognita* and *M. javanica* or during the emergence of new roots. It colonised the vascular bundles causing necrosis and preventing water and nutrient uptake. The vascular necrosis, unilateral initially, extended to the leaf vines of apical twigs resulting in quick wilt and death of plants. Externally, diseased plants showed yellowing, shedding of leaves internodes and lack of rootlets. (Duarte, *et al*. 1999).

2.4.2 Burrowing Nematode (Radopholus similis)

Ridley (1912) observed that in pepper plantations in Wayanad, Kerala, many pepper plants died due to nematode disease after a period of phenomenal success in pepper cultivation. He also found a series of tumours (root galls) in plant tissue due to eel worms (*Heteroderaradicicola= Meloidogyne incognita*). Similarly, during 1930s in the Indonesian island, Bangka, pepper plants with foliar yellowing and defoliation were observed and the disease was termed as ‘yellows’ by Bregman (1940). Now this nematode disease of pepper with characteristic symptoms of foliar yellowing and defoliation is known as ‘slow decline’ for the sake of uniform terminology.

Pepper plants infested with *R. similis* expressed through above ground symptoms like foliar yellowing, defoliation, lack of vigour and retardation in growth.

Vecht (1950) correlated the occurrence of yellows disease characterized by foliar yellowing with *R. similis* infestation in Bangka, Indonesia. Similarly, in India also a high correlation was noticed between the foliar yellowing and infestation with *R. similis* in pepper plantations (Ramana *et al*, 1987). Freire (1982) found that *R. similis* predisposed pepper seedlings to a week pathogenic isolate of *F. solani* and root rot was more severe.

In the Kampot region of Cambodia, the pepper industry suffered heavily due to a nematode fungal complex disease and pepper population was reduced from 2.5 million in 1953 (Hubert, 1957). Crop loss estimated due to this disease in India was not available though. Menon (1949) reported about 10 per cent mortality of pepper in Kerala. Wahid and Sitepu (1987) reported that annual loss reached up to 10-32 percent. Almost all plantations in Indonesia were affected by the disease. According to them the symptoms of the disease were foliar yellowing and leaf fall in both young and older plants. The yellowing of leaf started from the bottom of the plant and spread to the top, covering the whole plant at later stages of the disease. They were also of the opinion that the disease was mainly due to plant parasitic nematodes, *R. similis, M. incognita* and the fungus *Fusarium sp*. combined with agronomic disorders.

R. similis invaded any succulent underground plant part but favours the area near the root tip. Nematodes took feeding position inter and intra cellualarly and the cortical cells immediately around the nematode turned necrotic and further feeding and movement of the nematodes in the root tissue lead to development of large necrotic lesions throughout the root cortex. Under artificial inoculation the nematodes penetrated the pepper roots within 24 hours (Venkitesan and Setty 1977). The nematodes starved to death in less than 6 months in the absence of a host plant. All stages of the nematode after hatching from egg, except the adult males, were infective. *R. similis* fed on cortical tissues and produced elongated dark brown necrotic lesions on the roots at the infection sites. After draining the cell contents,

nematode pushed through the cell wall to next cell wall, thus destruction of successive cells resulted in the formation of tunnels or burrows in the root tissues.

Mohandas *et al.*, (1985) reported a sedentary, semi-endoparasite, nematode belonging to the family of *Tylenchulidae*. The nematode was described from the roots of pepper in Kerala. Tylenchulidae was widely prevalent in all major pepper growing areas in Kerala and Karnataka (Ramana and Mohandas, 1987; Ramana and Eapen, 1997).

Yellows disease was reported as a major problem for pepper cultivation that has upset the economy of Bangka islands in Indonesia where millions of pepper plants died during 1950's (Christie, 1959). About 30 per cent plants were damaged by this disease in Guyana. (Biessar, 1969).

Yellows disease was one of the reasons for low productivity of pepper in Indonesia (Mustika, 1990). This disease was also widely prevalent in Johore and Sarawak in Malaysia, and yield losses ranged from 25-90 per cent and the life span of the vine was reduced to 8-10 years (Varughese and Anuar, 1992)

Slow decline was reported as a debilitating disease over a period of time. The above ground symptoms of the disease were yellows, defoliation, die-back, loss in vigour and productivity, leading to slow death. On roots, nematode infestation resulted in the formation of galls due to root-knot nematodes, necrotic lesions and rotting caused by *R. similis* resulted in total loss of feeder roots. Infested plants sometimes recovered with the onset of monsoon when the plants put forth new roots and leaves. However, the plants succumbed to the disease as the root generation could not compensate the root loss due to nematode damage (Mohandas and Ramana, 1987).

This disease was primarily attributed to *R. similis* or *Meloidogyne sp*. in all the pepper growing countries (Christie 1957, Ting 1975, Nambiar and Sarma, 1977,

Venkitesan and Setty, 1977; Mustika and Zainuddin, 1978; Ramana *et al*, 1987). However, there were different opinions on the etiology of the disease. Hubert (1957) and Bridge (1978) were of the view that though *R. similis* was primarily responsible for the disease, combined infestation of *Fusarium solani* along with the nematode resulted in the yellows disease. Infestation by the nematode and fungus together enhanced the root damage and severity of foliar yellowing (Lopes and Lordello 1979, Freire 1982, Hamada *et al*. 1985). Mustika (1990), in a pot culture test, observed that *R. similis* alone could cause yellowing of leaves with stiff droop but these symptoms were more severe when plants were inoculated with *R. similis* along with *M. incognita* or *F. solani* thus indicating that pepper was more affected by *R. similis* than *M.incognita* causing more root damage and thereby severe growth inhibition.

Pathogensity trials conducted in micro plots under simulated filed conditions in India confirmed that *R.similis* caused more damage to pepper than *M.incognita* (Mohandas and Ramana 1991).The possible role of *Fusarium spp*. in the disease complex is not elucidated in the large scale field trials conducted in India (Ramana *et. al*., 1992). It was also reported that both *R.similis* and *M. incognita* were mutually suppressive under greenhouse experiments (Eisenback, 1985).

In India, *P.capsici* was reported as a major constraint in black pepper cultivation. Roots of diseased plants showed infestation of *R. similis, M. incognita* and *P. capsici* either alone or in combination and there was no spatial segregation under field condition. Feeder roots damage caused by *P. capsici* was reported to lead to slow decline symptoms (Anandaraj *et al.* 1996; Ramana *et al*.1992).

Mustika (1992) reported that single or combination effects of *Radopholus similis* and *Fusarium solani* have been studied on black pepper. *Radopholus similis* significantly reduced plant height, number of nodes, length of nodes, number of leaves, leaf area, shoot and root weight. *Fusarium solani* also caused such reductions, but to a lesser extent than did *R. similis*. The combination of *R. similis* and *F. solani*

caused the same symptoms. *Radopholus similis* alone caused growth reduction and yellow leaves with stiff droop, but the damage was more obvious when *R. similis* acted together with *F. solani*.

Fourteen genera of plant parasitic nematodes were associated with black pepper (*Piper nigrum* L.) in nine major districts of Kerala. The concomitant infestation of *M. incognita*, *R. similis* and *T. piperis* in the roots of black pepper was high when associated to their solitary infestation. It was observed that the disease caused by fungi developed faster and become aggravated in presence of plant parasitic nematodes (Ramana and Mohandas, 1987).

The damage caused by *M. incognita* alone was less but in combination with *R.similis* and *P.capsici* the damage was non synergistic. So, an integrated approach to check all the three pathogens was essential for the management of slow decline disease (Anandaraj *et al*. 1996).

The nematode penetrated only 3-4 cell layers deep in the roots and necrotic lesions developed at the feeding sites. The infested roots also showed shrinkage and drying at the site of infection. Besides pepper, *Glyricidia sepium* and *Artocarpus heterophyllus*, which were used as live standards for trailing pepper, were also reported as hosts of this nematode (Ramana and Eapen, 1997).

Thuy *et al*, (2012) conducted survey in nurseries and plantations of black pepper plants in Quang Tri province in Vietnam. During the rainy season of 2007, nine fungal taxa were isolated from the roots of black pepper plants. *Fusarium solani* was found in about one out of four black pepper root samples examined but not in the nurseries and not from black pepper plants younger than five years growing in plantations. Since in these nurseries about one out of two black pepper plants examined had yellow leaves, this observation suggested that another pathogen must be the initial cause of the yellowing of the leaves. A likely pathogenic candidate is *M.*

incognita which was extracted from every single black pepper plant examined in the nurseries.

MacGowan (1982) reported that burrowing nematode had been associated with diseased black pepper vines in Kerala, India. Large numbers of nematodes were found infecting the roots of black pepper vines showing symptoms of a disease called slow wilt. From the roots of pepper vines afflicted with slow decline disease, *R.similis* was isolated and cultures were multiplied on pepper and banana (Musa sp.). Rooted black pepper cuttings with uniform stem girth, node length and leaf and lateral root numbers were potted and inoculated with 10, 100, 1000, 10,000 nematodes each in replication of eight. Some of the plants inoculated with 1000 and 10,000 nematodes began wilting after 90 days and were dead after 118 days. Shoot growth had been reduced 72-90 per cent. Leaves reduced by half in number, were smaller in size. No yellowing of the leaves of inoculated plants was observed. In general, stunting increased as inoculum levels increased.

Ichinohe (1976) reported that in Indonesia "Yellows" disease due to *Radopholus similis* was rare, but heavy infestations of root knot nematode, *Meloidogyne incognita* were widely distributed and were associated with a dense yellowish discolouration of the leaves and stunted growth. They observed that *Fusarium oxysporum* caused or intensified the disease on cotton, tomato, tobacco, watermelon, cucumber, and cabbage in association with *Meloidogyne incognita.*

Ramana *et al*. (1987) surveyed nine districts of Kerala and reported that high populations of *Radopholus similis* occurred more frequently in disease affected vines (4 out of 5) than in healthy vines. High populations of *Meloidogyne incognita* and *Trophotylenchulus piperis* occurred in equal frequency in healthy and diseased vines. The results also indicated that *R. similis* played a major role in causing slow wilt disease in black pepper.

Mohandas and Ramana (1988) reported that the population of *R. similis* reached maximum in the month of September/October and minimum in the month of April/May and was detectable throughout the year. Black pepper was found to harbor more nematodes per gram of root compared to citrus, banana, coconut or areca nut and rainfall and temperature influenced the nematode population.

Mustika (1992) reported that single or combination effects of *Radopholus similis* and *Fusarium solani* have been studied on black pepper *cv.* Kalluvalli. *Radopholus similis* significantly reduced plant height.

Ravindra *et al*. (2014) reported that among plant parasitic nematodes, root knot nematode *Meloidogyne incognita* was one of the important limiting factors in production and productivity of black pepper in various districts of Karnataka. Further, it was involved in creating disease complexes along with fungi apart from inflicting the disease on its own. The maximum mean root knot nematode (3.52) was observed in Udupi district followed by Shimoga (3.58) and least mean RKN was observed in Kodagu district (92.73). Further in all the districts, fungal nematode associations were observed leading to slow wilt complex in pepper.

Random survey was conducted and six soil samples were collected from pepper rhizosphere from different locations of taluks *viz.*, Thodapuzha, Devikulam, Udumbanchola and Peermedu of Iddukki district to determine the present status and distribution of plant parasitic nematodes (PPN) associated with black pepper Total of nine PPN were found in all taluks. Among them, six PPN were found each in Thodapuzha, Devikulam and Udumbanchola, whereas four in Peermedu. Plant parasitic nemeatodes were most frequent, abundant and prominent in Devikulam and Thodpuzha taluk, while less in Peermedu taluk. A minimum of one and a maximum of five PPN were recorded in a location. *R. similis* was the most frequent, abundant and prominent in Peermedu and less in Udumbanchola taluk (Pervez *et al*., 2016).

The nematode was found in more number in acidic soils (pH 4.6 to 6.0) Pervez *et al*., 2016).

RKN infested plant leaves exhibited dense yellowish discolouration, root system became heavily galled, egg masses with females enclosed deep within roots, galls were smooth and bigger sized in a few cultivars but small galls in many cultivars. While, the most diagnostic RKN damage occurred below ground, numerous symptoms could also be observed above ground. Severely affected plant leaves showed yellowing, chlorosis and stunting in the fields, resulted crop yields were reduced. Root-knot nematode population in roots of pepper reached maximum during April-May and minimum during December and January. A low soil temperature coupled with adequate soil moisture availability of fresh tender roots helped in the buildup of its population during September- October. The nematode was detectable throughout the year. Various factors like, rainfall and temperature influenced nematode populations (Rashid *et al*. 2017).

The nematodes produced small, elongated lesions on the young tender roots, and later these lesions coalesced and caused extensive root rotting. The primary symptoms were pale yellow, whitish, discoloration of leaves, typical orange to purple coloured lesion on young roots, root system exhibited extensive rotting, main roots were devoid of finer roots, that rot quickly, extensive necrosis of longer lateral root developed yellow patches that later turned as barren standard that had lost their vines or standard supporting dead vines without any leaves. These symptoms were well pronounced when soil moisture was depleted. In general, foliar yellowing and defoliation were low during July and high during April- May (Pervez, 2018).

2.5 ANALYSIS OF RHIZOSPHERE FOR ROOT MEALY BUGS

Devasahayam *et al*. (2009) conducted surveys in 297 gardens in 99 locations in Kerala and Karnataka in India showed that five species of mealy bugs infested the roots and basal region of stem (under the soil) of black pepper vines (*Piper nigrum*

L.). Infestation was observed in all the taluks surveyed in Wayanad (Kerala) and Kodugu (Karnataka) taluks and in Udumbanchola, Kozhikode, Taliparamba, (Kerala), Alur and Sakelshpur (Karnataka) taluks. The infestation was positively and significantly correlated with altitiude and was observed in all cultivars /varieties, and on vines trailed on all standards (support tress), resulting in defoliation, yellowing and wilting of leaves and mortality of vines. *Phytopthora capsici*, *Meolidogyne incognita* and *Radopholus similis* were associated with root mealy bug infested vines.

Najitha (2016) reported that, the study on population dynamics of root mealy bugs showed highest root mealybug population in cooler months (November to January) and lowest population in rainy months (June and July). Ants were responsible for spread of root mealy bugs.

2.6 INFLUENCE OF WEATHER VARIABLES ON YELLOWING

Relatively high rainfall during the monsoon with high soil moisture (>25%) and favorable temperature (22ºC to 29°C) and proper relative humidity (80%) were favourable for rapid multiplication of the fungus, phytophthora which is moisture loving (Anandaraj *et al*., 1996; Bong and Saad 1985).

Vijayakumar *et al*. (1985) reported that even with favourable soil moisture conditions, the leaves of plants (cv. Panniyur1) exposed to direct solar radiation developed symptoms of physiological disorder, *i.e*. yellowing followed by the formation of necrotic patches. The chlorophyll content of exposed leaves was 44 % below the content of shaded leaves (about 2.2 mg/g fresh weight).

Productivity of black pepper depended on, temperature, rainfall, elevation, soil fertility, cultural practices, age of the crop and climatic conditions during flowering, fruit set and development significant relationships with black pepper yield (Sivaraman *et al*. 1999).

Yudiyanto *et al*. (2014) assessed that environmental factors were affecting productivity of black pepper, like rainfall intensity, light intensity and micro humidity was most influencing factor in black pepper plantation area in Lampung province (Indonesia).

Rainfall and relative humidity positively affected the leaf and root production of black pepper varieties. Whereas pepper production declined due to increase in temperature and bright sunshine hours. (Sushna and Ajithkumar, 2017).

Materials and methods

3. MATERIALS AND METHODS

The present study entitled, "Investigations on yellowing of black pepper (*Piper nigrum* L.)" comprising laboratory and field experiments was carried out during the period 2016-2019 at Department of Plantation crops and Spices, College of Horticulture, Radio Tracer Laboratory, College of Horticulture, Department of Plant Pathology, College of Horticulture and Banana Research Station, Kannara. Part of laboratory analysis was also conducted in the nematology lab at IIHR, Bangalore and ICAR-Central Potato Research Station in Ooty. The study was undertaken to find out the role of soil nutrients, plant pathogens, insects and nematodes if any in causing yellowing of black pepper. The details of the methodologies followed during the course of the experiment are given below.

3.1 IDENTIFICATION OF BLACK PEPPER PLANTS SHOWING YELLOWING

Location and climate

The experimental fields were selected at College of Horticulture, Vellanikkara and from farmer's fields in Thrissur district. Survey was conducted based on disease spread and intensity of yellowing in black pepper gardens. Fields were selected from those areas having maximum concentration of general yellowing.

The experimental area was influenced by a typical warm humid tropical climatic condition and benefitted by southwest and north east monsoons. The experimental area received maximum amount of rainfall during the months of May, June, July and August. The mean maximum and minimum temperatures of the study location recorded were 33 ^{o} C and 23.3^{o} C respectively. The mean relative humidity was 74 per cent.

Plate 1 General outline of field view

Table 1. Locations of survey for collection of disease samples of black pepper

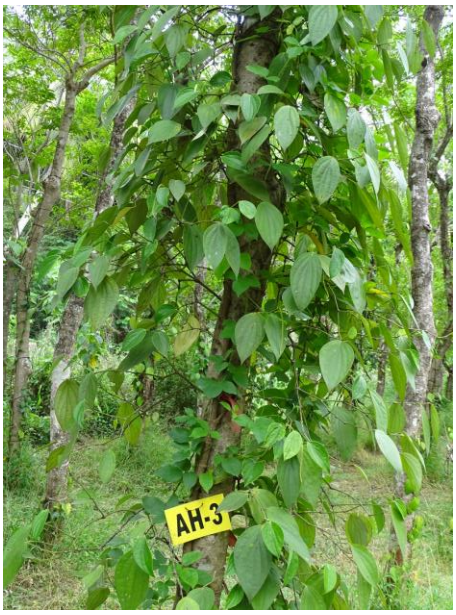
Sl. No.	Thrissur locations	Seasons (2017-2019)	Tagged plants
1.	Black pepper research unit, Dept. of Plantation Crops & Spices, College of Horticulture	July-August (Season-1) October-November (Season-2) February-March (Season-3)	Yellowing affected plants Apparently healthy plants
2.	Gokhale Block, Dept. of Plantation Crops & Spices, College of Horticulture	July-August, (Season-1) October-November (Season-2) February-March (Season-3)	Yellowing affected plants Apparently healthy plants
3.	Ameena. K. K Chirakakkode, (Farmers field	July-August (Season-1) October-November (Season-2) February-March (Season-3)	Yellowing affected plants Apparently healthy plants
4.	Joseph. P Vaniyampara, (Farmers field	July-August (Season-1) October-November (Season-2) February-March (Season-3)	Yellowing affected and apparently healthy plants
5	N. Bose Chelakkara, (Farmers field)	July-August (Season-1) October-November (Season-2) February-March (Season-3)	Yellowing affected plants Apparently healthy plants
6	Dr. Anitha. M University Nagar, Mannuthy, (Farmers field)	July-August (Season-1) October-November (Season-2) February-March (Season-3)	Healthy plants only

Table 2. Weather data of Thrissur district

Month	Maximum Temperature (^{o} C)	Minimum temperature (^{o} C)	Relative humidity (%)	Rainfall (mm)
2017				
June	30.4	23.5	87	630.2
July	30.8	22.8	85	385.5
August	30.1	23.3	87	478
September	31.5	22.9	84	413.91
October	31.7	22.3	81	183.4
November	33	21.8	73	58.3
December	32.4	21.1	63	11.5
2018				
January	33.5	20.9	53	0
February	35.7	22.5	47	5.2
March	36.7	24	59	33.2
April	36.1	24.8	69	28.9
May	33.2	22.6	79	483.6
June	29.8	23.8	89	730
July	29.6	22.5	88	793.2
August	29.2	22.2	87	928
September	32.2	22.5	75	290
October	32.8	22.2	76	393
November	32.7	22.9	68	66
December	33	23.3	63	0
2019				
January	32.9	20.4	55	0
February	35.8	23.4	59	0
March	36.7	24.8	65	0

Plate 2. Symptoms of different categories of plants selected for study

Yellowing affected plants



Apparentley healthy plants

Healthy plants

Plate 3. Survey and identification of yellowing at farmers fields

Farmers field at Chelakkara

Farmers field at Chirakkakode

Farmers field at Vaniyampara

Farmers field at university Nagar

Plate 4. Field survey and tagging of black pepper plants in farmers field

The total rainfall was 2360.6 mm. The place is situated at 10 ^{0} 32' N latitude and 76 ^{0} 13' E, with an altitude of 22.5 m above mean sea level. Table 2 shows the details of monthly weather parameters during the experimental period.

3.2 SELECTION OF EXPERIMENTAL PLANTS

A comprehensive purposive sampling survey was conducted in the pepper growing tracts of Thrissur so as to initially identify the intensity and spread of yellowing. Based on the survey, six different fields were selected for conducting the experiment.

In the selected tracts, samples were specifically identified and collected in a scale that included a. healthy, b. apparently healthy and c. yellowing affected plants. Fifteen yellowing affected plants and fifteen apparently healthy plants were selected from the same field. Healthy plants were selected from fields without disease symptoms. Black pepper varieties like Panniyur 1, Panniyur 2, Panniyur 3, Karimunda, and Vijay were included in the study. The locations of experiment are given in table 1.

3.3 DETAILS OF EXPERIMENTS

3.3.1 Experiment-1

Study on the initiation and development of yellowing

Plants showing initial symptoms of yellowing were tagged and analysed for symptoms, development of yellowing and its influence on yield.

Symptomatological studies

In the selected fields, plants were tagged and analysed for development of symptoms of yellowing at regular intervals and its influence on yield

Per cent disease severity

The per cent disease severity (PDS) was assessed in case of all foliage diseases following a standard score chart of 0-9 scale as depicted in Table 4. The PDS was calculated using the formula by Raja-Kumar *et al*. (2012)

$$\textbf{Per cent disease severity (PDS)} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of leaves observed x Maximum disease grade}} \times 100$$

Design: Factorial (3x2x3)

Number of treatments: 3

Replications: 15

Yield and yield contributing characters

Observations were taken on yield and yield contributing characters such as variety, plant spread in North-South and East-West directions, height of bearing column, number of laterals per column, number of spikes per column, pedicel length, length of spike, number of berries per spike, number of pin heads per spike, 100-berry weight, 100-berry volume and total green berry yield. Yield parameters were recorded at the time of harvest during the study in 2017-2019.

Observations on black pepper

Height of bearing column (m)

Calibrated iron pole was used to record the height of bearing column of pepper vine

Number of laterals and spikes per 0.25 m^2

With the help of a square wooden frame having 0.25 m^2 area, the spike bearing laterals and number of spikes were counted at chest height on all the four sides of the vine.

Plant spread

The spread of plant in North-South and East-West, directions were measured using measuring tape and expressed in cm.

Spike characters

At the time of harvest, ten spikes were randomly selected from each tagged plant, pedicel length, spike length, number of berries per spike and numbers of pin heads per spike were counted and average was calculated.

Green berry yield per vine

Immediately after harvest, total yield of green berries per vine was recorded. Yield of tagged plants in the healthy, apparently healthy yellowing affected groups were recorded for two consecutive years.

Varietal reaction/susceptibility to yellowing

Black pepper varieties Panniyur 1, Panniyur 2, Panniyur 3, Karimunda and Vijay were evaluated in the study, for per cent severity of yellowing and influence of yellowing on yield and yield contributing characters.

3.3.2 Experiment-2

3.3.2.1 Analysis of rhizosphere soil characters and their association with yellowing

Design: Factorial (3x2x3)
Number of treatments: 3
Replications: 15

Collection and processing of soil samples

Soil samples were collected from the different locations selected during the three different seasons. (July-August, October-November and February-March). Altogether 45 samples were collected in separate polythene bags tied with rubber band and labeled. The soil samples were air dried under shade, ground and sieved through 2

mm sieve and used for characterisation with respect to pH, EC, organic carbon and available nutrient status of (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Al). The procedure followed for characterization of soil samples are detailed here under.

Electrochemical properties of soil

Soil pH

Soil- water suspension in the ratio 1: 2.5 was prepared for determining soil pH potentiometrically using a pH meter (Jackson, 1958).

Electrical conductivity

Electrical conductivity was estimated in the supernatant liquid of the soil water suspension (1:2.5) used for the pH estimation with the help of a conductivity meter (Jackson, 1958).

Organic carbon

Organic carbon was determined by wet digestion method proposed by Walkley and Black (1934).

Available nitrogen

Modified Kjeldhal's method (Jackson, 1958) was followed to estimate nitrogen, Monoacid digestion using sulfuric acid and digestion mixture followed by distillation using Micro Kjedahl's distillation apparatus was carried out.

Available phosphorus

Available phosphorus was determined by extracting with Bray No. 1 reagent and estimating calorimetrically by reduced molybdate ascorbic acid blue colour method using spectronic 20 spectrometer (Bray and Kurtz, 1945).

Available potassium

Available potassium were extracted with neutral normal ammonium acetate solution and their contents determined by flame photometry (Jackson, 1958).

Available calcium and magnesium

Available calcium and magnesium in the soil samples were extracted using neutral ammonium acetate and its content in the extract was estimated using Atomic Absorption Spectrophotometer (Model; Perkin Elmer A Analyst 400).

Available micronutrients

Available micronutrients in soil samples were estimated by shaking four gram soil with 40 ml of 0.1 M HCl for 5 minutes. It was filtered through Whatman No. 42 filter paper and the filtrate was collected and analysed for Fe, Mn, Zn and Cu using Atomic Absorption Spectrophotometer (Model: PerkinElmer –PinAAcle 500).

Available sulphur

Available Sulphur was extracted by using 0.15% $CaCl_2$ (Tabatabai, 1982) and estimated by turbidimetry (Massoumi and Cornfield, 1963) using a spectrophotometer (Model: Systronics 169).

Available boron

Available boron in soil samples were extracted with hot water (Berger and Truog, 1939 and Gupta, 1977) and estimated calorimetrically by Azomthine-H using spectrophotometer (Model: Systronics 169)

Available aluminium

Four grams of soil samples were taken in a centrifuge tube and 40 ml of 0.1 M $BaCl_2$ solution was added. It was shaken for two hours and filtered through Whatman

No. 42 filter paper. The extract was used for aspiration to inductively coupled plasma emission spectrometer (Model: Perkin Elmer-Optima 8000) for determination of Aluminium.

3.3.3 Experiment-3

3.3.3.1 Tissue analysis for nutrients

First mature leaves of laterals were collected from healthy, apparently healthy and yellowing plants. These were analysed for the nutrient content.

Design: Factorial (3x2x3), Number of treatments: 3, Replications: 15

Nutrient analysis of leaves of black pepper leaf samples

First mature leaves of laterals were considered as index leaves. Leaf samples of black pepper were collected following the procedure suggested by De Waard (1969). The leaf samples were cleaned, first dried under shade and then dried in a hot air oven at 70 ^{0} C, powdered and stored in plastic bottles for analysis. Leaf samples were analysed for N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Al. During three different seasons viz., July-August, October-November and February-March (Table 3).

Table 3. Analytical methods followed in plant analysis

Parameters	**Method**	**Reference**
Nitrogen	Micro-Kjeldahl distillation after digestion in H_2 SO_4.	Jackson, 1958
Phosphorus	Nitric-perchloric (9:4) di acid digestion and colorimetry using vanado-molybdo phosphoric yellow colour method.	Piper, 1966
Potassium	Nitric-perchloric (9:4) di acid digestion of plant sample followed by filtration and flame photometry determination	Piper, 1966
Calcium, Magnesium	Nitric-perchloric (9:4) di acid digestion followed by filtration. The filtrate was collected and analyzed for Ca and Mg using atomic absorption spectrophotometer (DOA, 2013).	Jackson, 1958
Sulphur	Nitric-perchloric (9:4) di acid digestion and Turbidimetry method	Tabatabai and Bremner,1970

Contd. Table 3

Iron, Manganese, Zinc, Copper	Nitric-perchloric (9:4) di acid digestion followed by filtration. The filtrate was collected and analyzed for Fe, Mn, Zn and Cu using Inductive Coupled Plasma Optical Emission Spectrometer (ICP-OES; Model: Perkin Elmer-Optima 8000).	Piper, 1966
Boron	Dry ashing at 5500 C in silica crucibles followed by extraction of ash in 10 ml of 0.36 N H2SO4 for one hour at room temperature and filtration through Whatman No.42 filter paper. Filtrate was used for B determination by colorimetric Procedure using Azomethine-H	Bingham, 1982
Aluminum	Extraction using 0.1 M Ba Cl2 by using ICP-OES (Perkin Elmer-model Optima 8000).	Hendershot and Duquette 1986

3.3.4 Experiment-4

3.3.4.1 Study on association of plant pathogenic organisms with yellowing

Rhizosphere soil from healthy, apparently healthy and yellowing affected plant samples were collected in July-August, October-November and February-March and to study the role of plant pathogenic organisms associated with yellowing.

Design: Factorial (3x2x3), Number of treatments: 3, Replications: 15

Per cent disease incidence

The per cent disease incidence was calculated by recording the number of plants showing symptoms of yellowing, out of the total number of plants in each location.

The disease intensity of yellowing affected plants was calculated by estimating the disease index using the simplified method described by Wheeler (1969).

Per cent disease incidence (PDI) = $\frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$

Per cent disease severity

The per cent disease severity (PDS) was assessed in case of all foliage diseases following a standard score chart of 0-9 scale as depicted in Table 4. The PDS was calculated using the formula by Raja-Kumar *et al*. (2012)

Table-4. Score chart for assessing the severity of yellowing

Grade	Description
0	No symptom
1	</=1 per cent leaf area shows yellowing
3	>1-10 per cent leaf area shows yellowing
5	>10-25 per cent leaf area shows yellowing
7	>25-50 per cent leaf area shows yellowing
9	>50 per cent leaf area shows yellowing

Per cent disease severity (PDS) = $\frac{\text{Sum of all numerical ratings}}{\text{Total no. of leaves observed x Maximum disease grade}} \times 100$

Total soil microflora from rhizosphere soil

Soil samples with sufficient moisture collected from rhizosphere region of pepper vines were utilized for the estimation of soil microbial population. Serial dilution plate technique (Jhonson and Curl, 1972) was followed. The details are given table 5.

Procedure of serial dilution technique

Ten gram of soil sample was transferred under aseptic conditions in to 250 ml conical flasks containing 90 ml sterilized distilled water and the contents were mixed by shaking for five minutes. One ml of the aliquot was taken and transferred to 9 ml water blank containing sterile distilled water. The suspension was then shaken for one minute for homogenization before further dilution. Dilutions up to 10^{-8} were prepared

for the isolation of microorganisms of specific groups. Dilutions were standardized for each media and its corresponding microorganism. One ml of the respective dilution was pipetted out and transferred aseptically into sterile petri dishes. Twenty ml of molten and cooled agar media was poured into the petri dishes. The plates were rotated clockwise and anti-clockwise manually for the uniform mixing of the aliquot with the agar media. The mixture was then allowed to solidify and incubated at room temperature in inverted position. The number of colonies in the respective agar media were observed, recorded and calculations were made to obtain the number of colony forming units per gram of soil (cfu/g) from each category of soil samples. Observation on growth was taken at intervals of 24 h for a period of 4 days. Based on the observations done from the dilution and plating, alterations in the dilutions were made to obtain optimum number of colony forming units (Table 5). One ml aliquots from the dilution 10^{-3} and 10^{-5} and 10^{-8} were transferred to sterile petri plates. Melted and cooled media at 45 ^{o} C was poured at 20 ml per dish and rotated gently for thorough mixing. The petridishes were then incubated at 28 ^{o} C for 96 hours. Observations were recorded as number of colony forming units (cfu) per gram of soil.

Table 5. Media used for serial dilution and plate count for the isolation of microorganisms.

S l No.	**Microorganism**	**Media**	Dilutions
1	Fungi	Martin's Rose Bengal agar	10^{-3}
2	Actinomycetes	Kenknight' s agar media	10^{-5}
3	Bacteria	Nutrient agar	10^{-8}

3.3.5 Experiment-5

3.3.5.1 Analysis of rhizosphere soil and root samples for nematodes

During the three seasons, July-August, October-November and February-March, rhizosphere soil and root samples from a) healthy, b) apparently healthy and c) yellowing affected plants were collected and standard nematode extraction techniques

viz. Cobb's sieving and decanting methods were done. The collected nematodes were identified and per cent nematode population worked out.

Design: Factorial (3x2x3), Number of treatments: 3, Replications: 15

Soil sampling

The sampling was done during three seasons in a year *viz*. July-August, October-November and February-March (during 2017-2018 and 2018-2019). The samples were collected manually with the help of an auger from a depth of 10-20 cm, depending upon soil moisture. These composite samples were put in polybags and tied with rubber band to check the loss of moisture. Supporting data regarding soil temperature, altitude and locality, date of collection *etc*. were tagged to bag. The samples were brought to laboratory for further processing. In the laboratory, the samples were kept at 5^{o} C in order to maintain optimum moisture.

Nematode population in 250 cm^3 soil (number/250 cm^3 soil)

Soil samples were collected from rhizosphere of black pepper from different locations in Thrissur. Following the Cobb's decanting and sieving technique (Cobb' 1918). Out from composite sample, 250 cc of soil was weighed and thoroughly mixed with one liter of water in a pan. It was stirred well and the clods and clumps were broken. After 10-20 seconds, the soil suspension was passed to pan II through a 20 mesh sieve leaving heavy soil particles. The suspension of pan II was stirred gently, waited for 5-10 seconds and then poured through a series of 60, 100, 200 and 350 mesh sieves (mesh: number of apertures/linear inch) and the filtrates were discarded from 350 mesh sieve. The soil samples were processed, the nematode suspension thus obtained was made up to a constant volume (50 ml) by adding water. An aliquot of 5 ml was pipetted out in to a counting dish and the number of nematodes present was counted under stereo microscope. The total population of nematodes extracted from 250 cc soil sample was estimated by multiplying the average population with dilution factor.

Estimation of nematode population in 5 g root

Root samples consisting of primary and secondary roots and fibrous roots were collected from the fifteen black pepper vines. From each category of pepper plants to separate main and fibrous roots. Each type of roots were mixed thoroughly and three sub samples (5 g each) were taken from each sample for nematode analysis. Mean value was calculated for each of the three sub samples and the data were subjected to statistical analysis.

Estimation of root galls from roots

The root samples were collected from tagged plants in different locations. Roots were washed under tap water to remove excess soil from the roots. The root sample was pressed gently between folds of blotting paper to remove excess water and the number of root galls in 5g of root samples were counted.

Collection of juvenile stages

After washing root samples, each root gall embedded egg masses were collected and kept for incubation over night at 30^{0} C. Second stage juveniles (J_2) were harvested from the eggs at regular 24 hr intervals.

Collection of white females by staining technique

Root samples collected from the pepper plants were used for extracting white females. Roots samples were washed in a stream of tap water to remove any soil particles adhering to it. Root knots were separated from the roots with the help of scissors. It was then placed in small piece of muslin cloth and was wrapped in it. These small bags containing root knots were plunged in to boiling lacto phenol containing 0.1 per cent cotton blue for 3 minutes. The root knots were removed from muslin cloth and were kept in a petri plate. It was washed in water to remove excess stain. Glycerol was added to petri plate to remove excess stain and roots to become soft. It was kept in glycerol for 24 hours and then replaced with fresh glycerol. The

root knots were transferred to a microscopic slide containing a drop of lacto phenol. It was then placed under stereo microscope and was dissected using a needle. The white females, which were stained light blue, came out from root knots in large number, were collected and transferred to a glass vial, containing lacto phenol.

3.3.6 Experiment-6

3.3.6.1 Analysis of rhizosphere for root mealy bugs

Rhizosphere soil from a) healthy, b) apparently healthy and c) yellowing affected plants and root samples were collected in the month of October- November and February- March for studying the presence of root mealy bugs. Observations were made on presence or absence of root mealy bugs and intensity of mealy bug attack if any.

Design: Factorial (3x2x3), Number of treatments: 3, Replications: 15

3.3.7 Experiment 7

3.3.7.1 Influence of weather variables on yellowing

Meteorological data recorded by Department of Agricultural Meteorology, College of Horticulture, Vellanikkara were utilized to study the effect of weather parameters if any, on yellowing.

Correlation with weather variables

Weather data *viz*., maximum and minimum temperature, relative humidity and rainfall under field conditions were collected and recorded during survey period from the observatory maintained by Department of Agricultural Meteorology, College of Horticulture, Vellanikkara.

3.3.8 Statistical design

The design of the experiment was 3x2x3 factorial with 15 replications. The first factor was three seasons (July –August, October-November and February-March), the second factor was two years (2017-18 and 2018-19) and the third factor was category/disease status (healthy, apparently healthy and yellowing affected plants).

Results

4. RESULTS

4.1 EXPERIMENT I

Study on the initiation and development of yellowing

4.1.1 Symptomatological studies

Symptomatological studies on yellowing in black pepper showed development and spread of yellowing in pepper had varying patterns

Symptoms of yellowing generally developed at the fag end of monsoon. As it can be seen from (table 6) per cent severity of yellowing was least during monsoon. Per cent severity of yellowing was significantly higher during October-November (at fag end of monsoon) and was on par during summer. In case of mild yellowing developed at fag end of monsoon, it was observed that there was recovery during July-August and onset of monsoon. In all the three seasons yellowing affected plants showed significantly higher per cent severity of yellowing than apparently healthy plants, whereas, healthy plant showed significantly lower intensity yellowing.

Yellowing was observed either at the top of pepper column or (and) the bottom of the column. Sometimes yellowing appeared in a group of leaves together. In some aged plants older leaves at terminal portion of plant were yellow. In some leaves the base of lamina near petiole was yellow. This yellowing faded to tip of lamina and margins.

In some other cases, interveinal yellowing was observed. Sometimes yellowing was observed in one or two leaves on a branch where as in some other cases all the leaves on lateral branch were yellow.

Table-6 Per cent severity of yellowing during different seasons of yellowing affected plants, apparently healthy and healthy black pepper plants during study

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	30.46 (5.44)	24.53 (4.64)	**27.50 (5.04)**	38.39 (6.18)	35.30 (5.91)	**36.85 (6.05)**	35.74 (5.93)	33.32 (5.43)	**34.53 (5.68)**
A.H	5.76 (2.37)	15.72 (3.81)	**10.74 (3.09)**	29.00 (5.28)	22.92 (4.76)	**25.96 (5.02)**	20.64 (4.14)	31.89 (5.48)	**26.27 (4.81)**
Healthy	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.82 (1.03)	0.11 (0.76)	**0.47 (0.90)**	0.32 (0.85)	0.22 (0.81)	**0.27 (0.83)**
Year mean	**12.07** (2.84)	**13.42** (3.05)		**22.74** (4.17)	**19.45** (3.81)		**18.90** (3.64)	**21.81** (3.90)	
Seasonal mean	**12.74** (2.95)			**21.09** (3.99)			**20.36** (3.77)		

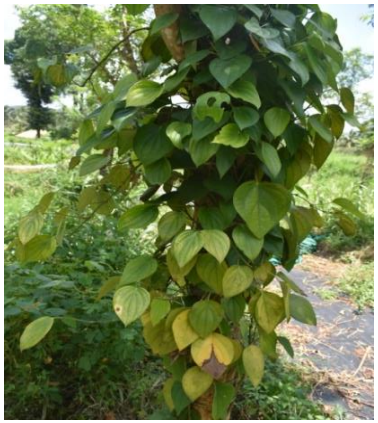
Category / Year (C)	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	34.86 (5.85)	31.05 (5.33)	**32.96 (5.59)**
Apparently healthy plants	18.47 (3.93)	23.51 (4.68)	**20.99 (4.31)**
Healthy plants	0.38 (0.86)	0.11 (0.76)	**0.24 (0.81)**
Category /Year mean	**17.90 (3.55)**	**18.23 (3.59)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.32**	Season x Year (A x B)	**0.45**
Year – (B)	**0.22**	Season x Category (A x C)	**0.56**
Category – (C)	**0.32**	Year x Category (B x C)	**0.45**
		Season x Year x Category (A x B x C)	**0.78**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Plate 5. Various symptoms of yellowing observed in experimental plants



Basal yellowing

Yellowing near petiole

Intense yellowing

Severe yellowing

Yellowing at crop maturity

Yellowing at middle of leaf blade

Plate 6. Various symptoms of yellowing observed in experimental plants

Pale yellow discoloration

Spread over all plant

Interveinal chlorosis

Severe interveinal yellowing

Yellowing at leaf tip

Intense yellowing

Another type of yellowing was observed at the time of fruit maturity during October-November. In this type of yellowing, recovery was observed with the onset of monsoon.

Yellowing started as mild discoloration of leaves which was spreading to whole plant. Yellowing was seen on leaves of all stages. Slowly the whole plant became yellow in colour. Sometimes yellowing started from leaf tip and spread to entire lamina. Sometimes yellowing was severe and leaves turned bright yellow.

Intensely affected lateral branches failed to grow further. There was no flushing or flowering in such branches. However, unlike fungal or insect attack there was no shedding of leaves. The lemon yellow coloured leaves remained on the plant.

In case of mild yellowing there was recovery during July-August with onset of monsoon. There was reduction in root nodules and fresh roots developed during monsoon. Yellowing gradually increased and severely affected plants failed to recover. There was no flushing or flowering in plants. Such plants died in 2-3 years.

4.1.2 Yield and yield contributing characters

Observations on yield and yield contributing characters were collected from fifteen spikes each in the category of yellowing affected, apparently healthy and healthy plants during 2017-18. The mean values for the characters in the different categories during 2017 and 2018 were worked out. The overall mean for the characters in the different categories were also calculated.

4.1.2.1 Height of bearing column

Height of bearing column did not show any significant difference in varieties Panniyur 3, Karimunda and Vijay (table 7). In Panniyur 2, height of bearing column was significantly higher in healthy plants compared to apparently healthy and yellowing affected plants, whereas in Panniyur 1 apparently healthy plants showed

lower height of bearing column, whereas healthy plants and yellowing affected plants were on par.

4.1.2.2 Plant spread N-S

Considering the spread of plant in North – South direction there was no significant difference among apparently healthy, healthy and yellowing affected categories in Panniyur 2, Karimunda and Vijay (table 8). In varieties Panniyur 1 and Panniyur 3 yellowing affected plants showed significantly lower spread in the North – South direction compared to healthy. Apparently healthy plants were on par with other two categories.

4.1.2.3 Plant spread E-W

In the case of spread in East-West direction, yellowing affected plants of Panniyur 1 and Panniyur 2 showed significantly lower spread compared to healthy plants (table 9). In all other varieties different categories were statistically on par.

4.1.2.4 Number of laterals per area

Analysis of number of laterals per unit area (0.25 m^2) did not show any significant difference among the different categories in variety Panniyur 3 (table 10). In varieties Panniyur 1, Panniyur 2 and Karimunda healthy plants showed significantly more number of laterals in a unit area compared to yellowing affected plants. Apparently healthy plants were on par with both healthy and yellowing affected plants in Panniyur 1 and Panniyur 2. In case of Vijay apparently healthy plants showed significantly lower number of laterals compared with healthy plants.

4.1.2.5 Number of spikes per unit area

Number of spikes per unit area did not differ significantly among yellowing affected, apparently healthy and healthy plants in Panniyur 1 and Panniyur 2. In varieties Panniyur 3, Karimunda and Vijay yellowing affected plants showed

significantly lower number of spikes in a unit area (0.25 m^2) when compared to healthy plants (table 11).

4.1.2.6 Green berry yield

Table 12 shows green berry yield per plant in the five varieties in the three categories of yellowing affected, apparently healthy and healthy plants. In varieties Panniyur 1, Panniyur 2, Panniyur 3 and Karimunda, there was significant reduction in yield in yellowing affected plants compared to healthy, whereas the significant difference between apparently healthy and yellowing affected plants was only in case Panniyur 1. In variety Vijay there was no significant difference in yield among the three categories.

4.1.2.7 Length of spike

Analysis of the length of spike showed that there was no significant difference in the length of spike among apparently healthy, healthy and yellowing affected plants (table 13).

4.1.2.8 Pedicel length

Pedicel length of yellowing affected, apparently healthy and healthy plants of varieties Panniyur 1, Panniyur 2, Panniyur 3, Karimunda and Vijay are given in table 14. Pedicel length was significantly lowest in yellowing affected plants of Panniyur2 and Vijay compared to healthy plants. Apparently healthy and healthy plants were statistically on par. In Karimunda apparently healthy and yellowing affected plants were on par for pedicel length and healthy plants showed significantly longer pedicel length. In Panniyur 1 and Panniyur 3 the three categories of plants were statistically on par for pedicel length.

Plate 7. Yield contributing characters

Plant spread East-West direction (cm)

Number of laterals per unit area (0.25 m^2)

Table 7. Height of bearing column (cm) of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	545.00	540.00	542.50	427.00	425.00	426.00	316.33	390.00	353.17	256.67	307.33	282.00	380.00	413.33	396.67
A.H	386.67	413.33	400.00	360.00	503.33	431.67	415.00	465.00	440.00	349.67	383.33	366.50	446.67	410.00	428.33
Healthy	731.67	583.33	657.50	584.33	660.00	622.17	450.00	470.00	460.00	410.00	398.00	404.00	431.33	466.67	449.00
Variety Mean	**554.44**	**512.22**		**457.11**	**529.44**		**393.78**	**441.67**		**338.78**	**362.89**		**419.33**	**430.00**	
Varieties	**533.33**			**493.28**			**417.72**			**350.83**			**424.66**		

CD (0.05)	CD (0.05)
Varieties	**79.839**
Variety x category	**138.286**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 8. Plant spread in North-South (cm) direction in yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	85.67	104.67	95.17	98.00	102.00	100.00	97.00	116.00	106.50	104.33	101.33	102.83	118.33	127.33	122.83
A.H	111.00	104.67	107.83	106.33	106.33	106.33	106.33	116.00	111.17	102.67	106.67	104.67	117.00	126.67	121.83
Healthy	123.33	120.67	122.00	110.00	110.00	110.00	126.67	125.33	126.00	105.67	125.67	115.67	123.00	130.00	126.50
Variety Mean	**106.67**	**110.00**		**104.78**	**106.11**		**110.00**	**119.11**		**104.22**	**111.22**		**119.44**	**128.00**	
Varieties	**108.33**			**105.44**			**114.56**			**107.722**			**123.722**		

CD (0.05)	CD (0.05)
Varieties	**11.986**
Variety x category	**20.761**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 9. Plant Spread East-West direction (cm) in yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	92.67	97.33	95.00	93.33	92.33	92.83	108.33	129.00	118.67	103.00	106.00	104.50	119.67	135.67	127.67
A.H	110.00	104.00	107.00	98.93	105.47	102.20	110.00	126.67	118.33	107.67	109.33	108.50	116.67	132.33	124.50
Healthy	129.33	125.00	127.17	124.00	121.33	122.67	137.00	135.33	136.17	107.67	134.33	121.00	120.00	137.33	128.67
Variety Mean	**110.67**	**108.78**		**105.42**	**106.38**		**118.44**	**130.33**		**106.11**	**116.56**		**118.78**	**135.11**	
Varieties	**109.72**			**105.90**			**124.39**			**111.333**			**126.944**		

CD (0.05)	CD (0.05)
Varieties	**12.488**
Variety x category	**21.630**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 10. Number of laterals per unit area (0.25 m^2) in yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	8.00	7.33	7.67	7.67	5.33	6.50	10.33	7.67	9.00	4.00	4.33	4.17	9.33	10.67	10.00
A.H	8.67	7.33	8.00	8.67	8.00	8.33	7.00	7.00	7.00	9.33	8.67	9.00	10.33	8.33	9.33
Healthy	10.00	10.00	10.00	8.67	9.33	9.00	10.33	8.00	9.17	10.33	12.00	11.17	12.33	11.00	11.67
Variety Mean	**8.89**	**8.22**		**8.33**	**7.56**		**9.22**	**7.56**		**7.89**	**8.33**		**10.67**	**10.00**	
Varieties	**8.56**			**7.94**			**8.39**			**8.111**			**10.333**		

CD (0.05)	CD (0.05)
Varieties	**1.323**
Variety x category	**2.292**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 11. Number of spikes per unit area (0.25 m^2) of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	18.67	10.67	14.67	15.67	18.67	17.17	10.00	18.67	14.33	11.00	5.00	8.00	21.67	24.33	23.00
A.H	18.67	14.33	16.50	10.67	17.00	13.83	23.00	18.67	20.83	13.67	14.00	13.83	21.00	35.00	28.00
Healthy	22.00	18.00	20.00	21.00	22.33	21.67	35.00	24.00	29.50	33.67	21.67	27.67	28.67	44.00	36.33
Variety Mean	**19.78**	**14.33**		**15.78**	**19.33**		**22.67**	**20.44**		**19.44**	**13.56**		**23.78**	**34.44**	
Varieties	**17.06**			**17.56**			**21.56**			**16.500**			**29.111**		

CD (0.05)	CD (0.05)
Varieties	**5.043**
Variety x category	**8.734**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 12. Green berry yield/plant (g) of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	1000.0	883.3	941.7	2533.3	1766.7	2150.0	2433.3	1900.0	2166.7	333.3	283.3	308.3	3100.0	3033.3	3066.7
A.H	2516.7	2033.3	2275.0	3000.0	2623.3	2811.7	3833.3	2933.3	3383.3	1530.0	1503.3	1516.7	3216.7	3133.3	3175.0
Healthy	6833.3	6270.0	6551.7	4166.7	4466.7	4316.7	4333.3	3533.3	3933.3	1700.0	1766.7	1733.3	3300.0	4000.0	3650.0
Variety Mean	**3450.0**	**3062.2**		**3233.3**	**2952.2**		**3533.3**	**2788.9**		**1187.8**	**1184.4**		**3205.6**	**3388.9**	
Varieties	**3256.1**			**3092.8**			**3161.1**			**1186.1**			**3297.2**		

CD (0.05)	CD (0.05)
Varieties	**785.152**
Variety x category	**1359.924**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 13. Length of spike (cm) of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	12.57	12.03	12.30	9.74	9.35	9.54	14.07	10.14	12.10	7.26	7.51	7.38	11.68	11.00	11.34
A.H	12.95	9.82	11.39	10.78	11.44	11.11	13.82	10.54	12.18	8.26	8.07	8.16	13.96	11.09	12.53
Healthy	13.98	12.48	13.23	11.22	11.57	11.39	14.80	10.88	12.84	10.00	7.22	8.61	13.95	11.29	12.62
Variety Mean	**13.17**	**11.44**		**10.58**	**10.79**		**14.23**	**10.52**		**8.51**	**7.60**		**13.20**	**11.13**	
Over all variety mean	**12.31**			**10.68**			**12.37**			**8.053**			**12.162**		

CD (0.05)	CD (0.05)
Varieties	**1.332**
Variety x category	**NS**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

4.1.2.9 Number of berries per spike

Number of berries per spike was significantly lower in yellowing affected plants of Panniyur 1 compared to apparently healthy and healthy plants (table 15). In the case of variety Karimunda yellowing affected plants showed significantly lower number of berries compared to healthy. In varieties, Panniyur 2, Panniyur 3 and Vijay the three categories were on par for number of berries per spike.

4.1.2.10 Number of pin heads per spike

There was no significant difference in the number of pin heads per spike in Panniyur 2, Panniyur 3 and Vijay (table 16). In Karimunda healthy plants showed significantly lower number of pin heads compared to apparently healthy and yellowing affected plants. In Panniyur 1, healthy plants showed significantly lower number of pin heads compared to yellowing affected plants.

4.1.2.11 Hundred berry weight

There was no significant difference in hundred berry weight of yellowing affected and apparently healthy plants in varieties Panniyur 2 and Vijay (table 17). In variety Panniyur 1 apparently healthy plants showed significantly lower hundred berry weight compared to healthy plants. In Panniyur 3 yellowing affected plants showed significantly lower 100 berry weight compared to healthy plants. In Karimunda yellowing affected plants showed significantly lower hundred berry weight compared to other two categories.

4.1.2.12 Hundred berry volume

There was no significant difference in hundred berry volume in the different categories in varieties Panniyur 3 and Vijay (table 18). In the variety Panniyur 1 and Panniyur 2 significantly low hundred berry volume was observed in apparently healthy plants compared to healthy plants. In the case of Karimunda yellowing

affected plants showed significantly lower hundred berry volume compared to apparently healthy and healthy plants.

4.1.3 Varietal reaction/susceptibility to yellowing

All varieties studied were found to be susceptible to yellowing. No definite pattern for incidence or spread of yellowing was observed in a plant.

4.2 EXPERIMENT II

4.2.1 Analysis of rhizosphere soil characters and their association with yellowing

For analysis of rhizosphere soil characters and to find out their association with yellowing, soil samples were collected from the rhizosphere of healthy, apparently healthy and yellowing affected plants from the selected fields. Samples were collected during, July-August and October-November 2017; February-March, July-August and October-November 2018 and February-March, 2019.

Soil samples were collected and carefully shade dried, sieved through 2 mm sieve and stored in polythene bags for further analysis of the nutrient elements present.

4.2.1.1 pH

The pH values of the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in (table 19). The pH of rhizosphere soil of healthy plants was significantly lower compared to apparently healthy and healthy. However, yellowing affected and apparently healthy plants were statistically on par. Comparing the seasons, the highest pH value (6.14) was observed during February-March, which was significantly higher than that in other two seasons. Soil pH was lowest (5.54) during October-November, which was significantly lower than the other two seasons.

Table 14. Pedicel length (cm) of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	1.38	1.38	1.38	1.26	1.22	1.24	1.50	1.31	1.40	1.41	1.02	1.22	1.28	1.25	1.27
A.H	1.32	1.39	1.36	1.41	1.52	1.47	1.64	1.46	1.55	1.20	1.06	1.13	1.36	1.41	1.39
Healthy	1.42	1.48	1.45	1.51	1.56	1.54	1.71	1.44	1.58	1.46	1.61	1.54	1.55	1.51	1.53
Variety Mean	**1.37**	**1.42**		**1.39**	**1.43**		**1.61**	**1.40**		**1.36**	**1.23**		**1.40**	**1.39**	
Varieties	**1.39**			**1.41**			**1.51**			**1.29**			**1.39**		

CD (0.05)	**CD (0.05)**
Varieties	0.137
Variety x category	0.238

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 15. Number of berries per spike of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	40.55	32.48	36.52	44.97	51.69	48.33	56.07	49.98	53.03	10.30	24.13	17.21	58.87	47.93	53.40
A.H	51.80	56.03	53.92	55.37	49.87	52.62	64.83	47.06	55.95	22.95	23.78	23.36	52.97	44.13	48.55
Healthy	56.47	57.23	56.85	61.76	55.77	58.76	69.33	54.16	61.74	33.57	26.63	30.10	60.33	51.27	55.80
Variety Mean	**49.61**	**48.58**		**54.03**	**52.44**		**63.41**	**50.40**		**22.27**	**24.85**		**57.39**	**47.78**	
Varieties	**49.09**			**53.24**			**56.90**			**23.55**			**52.58**		

CD (0.05)	CD (0.05)
Varieties	**7.373**
Variety x category	**12.770**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 16. Number of pin heads per spike of yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	35.11	27.50	31.30	22.87	19.19	21.03	22.42	15.87	19.15	26.23	30.03	28.13	23.77	22.21	22.99
A.H	18.68	25.56	22.12	21.57	16.57	19.07	18.37	16.50	17.43	27.85	26.53	27.19	24.20	17.98	21.09
Healthy	22.73	16.27	19.50	15.80	10.23	13.02	17.96	12.26	15.11	13.90	8.53	11.22	19.17	16.51	17.84
Variety Mean	**25.51**	**23.11**		**20.08**	**15.33**		**19.58**	**14.88**		**22.66**	**21.70**		**22.38**	**18.90**	
Varieties	**24.31**			**17.70**			**17.23**			**22.18**			**20.64**		

CD (0.05)	CD (0.05)
Varieties	**5.465**
Variety x category	**9.466**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 17. Hundred (100) berry weight (g) for yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	12.91	15.09	14.00	14.31	13.73	14.02	14.02	15.60	14.81	10.13	8.67	9.40	15.10	14.80	14.95
A.H	12.52	12.81	12.67	14.38	15.00	14.69	15.44	14.95	15.20	15.07	14.03	14.55	14.51	14.37	14.44
Healthy	15.57	15.57	15.57	14.67	15.48	15.08	16.67	16.33	16.50	15.20	14.49	14.85	15.67	15.27	15.47
Variety Mean	**13.67**	**14.49**		**14.45**	**14.74**		**15.38**	**15.63**		**13.46**	**12.40**		**15.09**	**14.81**	
Varieties	**14.08**			**14.60**			**15.50**			**12.930**			**14.954**		

CD (0.05)	CD (0.05)
Varieties	**0.928**
Variety x category	**1.608**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 18. Hundred (100) berry volume (cc) for yellowing affected, apparently healthy and healthy black pepper plants

Treatments	P1 2017	P1 2018	Pooled mean	P2 2017	P2 2018	Pooled mean	P3 2017	P3 2018	Pooled mean	Karimunda 2017	Karimunda 2018	Pooled mean	Vijay 2017	Vijay 2018	Pooled mean
Y	11.67	13.00	12.33	12.40	12.10	12.25	12.00	12.71	12.35	8.93	9.26	9.10	12.67	13.57	13.12
A.H	11.57	11.33	11.45	9.17	13.67	11.42	13.37	13.67	13.52	13.85	12.52	13.19	13.73	12.67	13.20
Healthy	13.17	14.00	13.58	13.33	13.83	13.58	13.67	13.51	13.59	14.67	13.28	13.97	14.73	13.67	14.20
Variety Mean	**12.13**	**12.78**		**11.63**	**13.20**		**13.01**	**13.30**		**12.48**	**11.69**		**13.71**	**13.30**	
Varieties	**12.46**			**12.42**			**13.15**			**12.09**			**13.51**		

CD (0.05)	
Varieties	**1.195**
Variety x category	**2.070**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

During July-August and February-March, the pH did not show significant difference in rhizosphere soils from yellowing affected, apparently healthy and healthy plants.

During October-November, soil pH was significantly lower in healthy plants compared to apparently healthy and yellowing affected plants. During February-March soil pH was significantly lower in the rhizosphere soil of healthy plants compared to apparently healthy plants. During July-August soil pH of healthy plants was significantly lower than that in apparently healthy plants.

4.2.1.2 Electrical Conductivity

The electrical conductivity of the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 20. The EC of the rhizosphere soil was on par in apparently healthy and yellowing affected plants. The EC was significantly lower in the rhizosphere soils of healthy plants. The highest EC value (0.17 dS m-1) was observed during February-March, which was significantly higher than other two seasons. Electrical conductivity was significantly lower (0.11 dS m^{-1}) during July-August, when compared with October-November.

During July-August, electrical conductivity was significantly lower (0.09 dS m^{-1}) in healthy plants compared to yellowing affected plants and apparently healthy plants. During October-November, electrical conductivity was significantly higher (0.14 dS m^{-1}) in rhizosphere soils of yellowing affected plants. Rhizosphere soils of healthy plants were significantly lower in electrical conductivity and was statistically on par with apparently healthy plants.

During February-March, rhizosphere soils of apparently healthy plants showed significantly high electrical conductivity (0.22 dS m^{-1}**).** Whereas, rhizosphere soils of healthy plants showed significantly lower (0.10 dS m^{-1}**)** electrical conductivity compared to yellowing affected plants.

Table 19. pH of rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	6.19	5.87	**6.03**	6.09	5.28	**5.68**	6.58	5.66	**6.12**
A. H	5.90	5.79	**5.85**	6.09	5.24	**5.67**	6.56	6.13	**6.35**
Healthy	6.14	5.33	**5.74**	5.55	5.00	**5.28**	6.42	5.50	**5.96**
Year mean	**6.08**	**5.67**		**5.91**	**5.17**		**6.52**	**5.76**	
Seasonal mean	**5.88**			**5.54**			**6.14**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	6.29	5.60	**5.94**
Apparently healthy plants	6.19	5.72	**5.95**
Healthy plants	6.04	5.28	**5.66**
Category /Year mean	**6.17**	**5.53**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.13**	Season x Year (A x B)	**0.19**
Year – (B)	**0.11**	Season x Category (A x C)	**0.24**
Category – (C)	**0.13**	Year x Category (B x C)	**0.19**
		Season x Year x Category (A x B x C)	**0.34**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 20. EC of rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.15	0.12	**0.14**	0.14	0.15	**0.15**	0.24	0.15	**0.20**
A. H	0.10	0.11	**0.11**	0.13	0.12	**0.13**	0.22	0.23	**0.22**
Healthy	0.09	0.09	**0.09**	0.11	0.11	**0.11**	0.12	0.09	**0.11**
Year mean	**0.12**	**0.10**		**0.13**	**0.13**		**0.19**	**0.16**	
Seasonal mean	**0.11**			**0.13**			**0.18**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	0.18	0.14	0.16
Apparently healthy plants	0.15	0.15	0.15
Healthy plants	0.11	0.10	0.10
Category /Year mean	**0.15**	**0.13**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.01**	Season x Year (A x B)	**0.02**
Year – (B)	**0.01**	Season x Category (A x C)	**0.02**
Category – (C)	**0.01**	Year x Category (B x C)	**0.02**
		Season x Year x Category (A x B x C)	**0.03**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 21. Per cent of organic carbon content in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	1.44	1.35	**1.39**	1.59	1.72	**1.65**	1.76	1.83	**1.79**
A. H	1.40	1.46	**1.43**	1.83	1.84	**1.83**	1.62	1.73	**1.67**
Healthy	1.04	0.51	**0.77**	0.87	0.98	**0.92**	1.59	1.63	**1.61**
Year mean	**1.29**	**1.10**		**1.43**	**1.51**		**1.66**	**1.73**	
Seasonal mean	**1.20**			**1.47**			**1.69**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	1.59	1.63	**1.61**
Apparently healthy plants	1.61	1.67	**1.64**
Healthy plants	1.16	1.03	**1.10**
Category /Year mean	**1.45**	**1.44**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.12**	Season x Year (A x B)	**0.18**
Year – (B)	**0.10**	Season x Category (A x C)	**0.22**
Category – (C)	**0.12**	Year x Category (B x C)	**0.18**
		Season x Year x Category (A x B x C)	**0.316**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 22. Content of nitrogen (kg ha^{-1}) rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	247.15	291.95	**269.55**	303.89	305.03	**304.46**	280.00	251.63	**265.81**
A. H	193.39	313.30	**253.34**	317.33	309.07	**313.20**	300.91	279.33	**290.12**
Healthy	318.08	314.20	**316.14**	311.36	319.20	**315.28**	321.07	321.81	**321.44**
Year mean	**252.87**	**306.48**		**310.86**	**311.09**		**300.66**	**284.26**	
Seasonal mean	**279.68**			**310.98**			**292.46**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	277.01	282.86	**279.94**
Apparently healthy plants	270.54	300.56	**285.55**
Healthy plants	316.83	318.40	**317.62**
Category /Year mean	**288.13**	**300.61**	

	CD (0.05)		CD (0.05)
Season- (A)	**12.99**	Season x Year (A x B)	**18.38**
Year – (B)	**10.61**	Season x Category (A x C)	**22.51**
Category – (C)	**12.99**	Year x Category (B x C)	**18.38**
		Season x Year x Category (A x B x C)	**31.836**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 23. Content of phosphorus (kg ha^{-1}) rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	124.94	146.83	**135.88**	164.45	150.68	**157.56**	135.16	136.13	**135.65**
A. H	106.50	81.40	**93.95**	103.93	108.64	**106.29**	79.06	97.13	**88.09**
Healthy	185.63	184.50	**185.07**	145.08	173.22	**159.15**	112.15	122.67	**117.41**
Year mean	**139.02**	**137.58**		**137.82**	**144.18**		**108.79**	**118.64**	
Seasonal mean	**138.30**			**141.00**			**113.72**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	141.51	144.54	**143.03**
Apparently healthy plants	96.49	95.72	**96.11**
Healthy plants	147.62	160.12	**153.87**
Category /Year mean	**128.54**	**133.46**	

	CD (0.05)		CD (0.05)
Season- (A)	**21.89**	Season x Year (A x B)	**30.96**
Year – (B)	**17.87**	Season x Category (A x C)	**37.92**
Category – (C)	**21.89**	Year x Category (B x C)	**30.96**
		Season x Year x Category (A x B x C)	**53.634**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 24. Content of potassium (kg ha^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	193.51	180.54	**187.03**	179.24	181.95	**180.59**	333.90	267.66	**300.78**
A. H	250.40	227.72	**239.06**	196.72	193.02	**194.87**	344.83	367.38	**356.10**
Healthy	293.95	281.26	**287.61**	287.09	296.37	**291.73**	469.68	353.54	**411.61**
Year mean	**245.95**	**229.84**		**221.02**	**223.78**		**382.80**	**329.53**	
Seasonal mean	**237.90**			**222.40**			**356.16**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	235.54	210.05	**222.79**
Apparently healthy plants	263.98	262.70	**263.34**
Healthy plants	350.23	310.39	**330.31**
Category /Year mean	**283.25**	**261.04**	

	CD (0.05)		CD (0.05)
Season- (A)	**29.71**	Season x Year (A x B)	**42.01**
Year – (B)	**24.25**	Season x Category (A x C)	**51.46**
Category – (C)	**29.71**	Year x Category (B x C)	**42.01**
		Season x Year x Category (A x B x C)	**72.77**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 25. Content of calcium (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	611.73	536.47	**574.10**	588.37	769.37	**678.87**	1169.67	934.03	**1051.85**
A. H	843.47	745.03	**794.25**	782.37	1002.34	**892.35**	1536.73	715.80	**1126.27**
Healthy	990.33	1217.53	**1103.93**	1229.27	847.88	**1038.57**	1937.30	1216.67	**1576.98**
Year mean	**815.18**	**833.01**		**866.67**	**873.19**		**1547.90**	**955.50**	
Seasonal mean	**824.09**			**869.93**			**1251.70**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	789.92	746.62	**768.27**
Apparently healthy plants	1054.18	821.05	**937.62**
Healthy plants	1385.63	1094.02	**1239.83**
Category /Year mean	**1076.58**	**887.23**	

	CD (0.05)		CD (0.05)
Season- (A)	**82.79**	Season x Year (A x B)	**117.08**
Year – (B)	**67.59**	Season x Category (A x C)	**143.39**
Category – (C)	**82.79**	Year x Category (B x C)	**117.08**
		Season x Year x Category (A x B x C)	**202.79**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 26. Content of magnesium (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	141.19	217.02	**179.11**	209.34	156.90	**183.12**	313.32	300.81	**307.06**
A. H	155.74	228.28	**192.01**	218.44	157.92	**188.18**	245.90	323.87	**284.89**
Healthy	203.05	242.89	**222.97**	228.95	136.01	**182.48**	240.91	358.42	**299.66**
Year mean	**166.66**	**229.39**		**218.91**	**150.27**		**266.71**	**327.70**	
Seasonal mean			**198.03**			**184.59**			**297.20**

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	221.28	224.91	**223.09**
Apparently healthy plants	206.69	236.68	**221.69**
Healthy plants	224.30	245.77	**235.03**
Category /Year mean	**217.42**	**235.79**	

	CD (0.05)		CD (0.05)
Season- (A)	**21.10**	Season x Year (A x B)	**29.85**
Year – (B)	**17.23**	Season x Category (A x C)	**36.55**
Category – (C)	**21.10**	Year x Category (B x C)	**29.85**
		Season x Year x Category (A x B x C)	**51.70**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

4.2.1.3 Organic carbon

The organic carbon content of the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 21. Organic carbon content in rhizosphere soils of healthy plants was significantly lower than yellowing affected and apparently healthy plants in the present study. The latter two categories were statistically on par. Organic carbon content was significantly higher during February-March compared to other two seasons. During July- August organic carbon content was significantly lower compared with October- November.

During July- August and October- November rhizosphere soils of healthy plants showed organic carbon content significantly lower than apparently healthy and yellowing affected plants. Whereas yellowing affected plants and apparently healthy plants were statistically on par. However, during February-March the three categories were statistically on par.

4.2.1.4 Nitrogen

The nitrogen content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 22. As can be seen from the table, over all nitrogen content during three seasons was significantly higher in the rhizosphere soils of healthy plants. Whereas, apparently healthy and yellowing affected plants were statistically on par.

In the rhizosphere soils, nitrogen content was significantly highest (310. 98 kg ha^{-1}) during October-November. The lowest nitrogen content was noticed during July-August (279.67 kg ha^{-1}) which was statistically on par with February-March (292.45 kg ha^{-1}).

During October-November, there was no significant difference in the nitrogen content in the rhizosphere soils of yellowing affected, apparently healthy and healthy plants. During February-March and July-August, nitrogen content showed significant

difference among the rhizosphere soils of yellowing affected, apparently healthy and healthy plants.

During February-March, healthy plant showed significantly high (321.44 kg ha^{-1}) nitrogen content. Yellowing affected plants showed significantly lower nitrogen in the rhizosphere soil compared to healthy and apparently healthy plants. During July-August, the rhizosphere soils of healthy plants showed significantly higher (316.13 kg ha^{-1}) nitrogen content when compared to apparently healthy and yellowing affected plants. Apparently healthy plants were statistically on par with yellowing affected plants.

4.2.1.5 Phosphorus

The phosphorus content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 23. As can be seen from the treatment mean phosphorus content was significantly lower in the rhizosphere soil of apparently healthy plants, whereas healthy and yellowing affected plants were statistically on par.

During February- March, phosphorus content was significantly lowest compared to October-November and July-August. The latter two were statistically on par.

In all the three seasons analyzed the rhizosphere soils of apparently healthy plants showed significantly lowest phosphorus compared to healthy and yellowing affected plants.

4.2.1.6 Potassium

The potassium content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 24. In the

rhizosphere soils of healthy plant potassium content was significantly higher compared to apparently healthy and yellowing affected plants.

The potassium content was significantly high (356.16 kg ha^{-1}) during February-March in the rhizosphere soil. The other two seasons were statistically on par.

During February-March and October-November, potassium content showed significant difference among the rhizosphere soils of yellowing affected, apparently healthy and healthy plants. During February-March, healthy plants showed significantly highest (411.61 kg ha^{-1}) potassium content when compared to rhizosphere soils of apparently healthy and yellowing affected plants. Yellowing affected plants showed significantly lower potassium content (300.78 kg ha^{-1}).

During October-November, the rhizosphere soils of healthy plants showed significantly higher (291.73 kg ha^{-1}) potassium content when compared to apparently healthy and yellowing affected plants. The other two categories were statistically on par. During, July-August the rhizosphere soils of yellowing affected plants showed significantly lower (187.03 Kg ha^{-1}) potassium content compared to other two categories, which were statistically on par.

4.2.1.7 Calcium

Calcium content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 25. The highest (1251.70 mg kg^{-1}) calcium content was observed in the rhizosphere soils of black pepper during February-March season, which was significantly higher than other two seasons. The calcium content during other two seasons were statistically on par.

The rhizosphere soils of healthy plants showed significantly highest calcium content compared to apparently healthy and yellowing affected plants. The calcium content was significantly lowest in rhizosphere soils of yellowing affected plants.

During, October-November and July –August the rhizosphere soils of healthy plants showed significantly highest calcium content than yellowing affected plants.

4.2.1.8 Magnesium

Magnesium content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 26. The treatment means showed that there was no significant difference in the magnesium content of healthy, apparently healthy and yellowing affected plants.

The highest (297.20 mg kg^{-1}) magnesium content was noticed during February-March, which was significantly higher than other two seasons. The lowest magnesium content (184.59 mg kg^{-1}) was noticed during October-November, which was on par with July-August. During the three seasons there was no significant difference in the magnesium content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

4.2.1.9 Sulphur

Sulphur content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 27. As can be seen from the table, sulphur content was significantly highest in the rhizosphere soils of healthy plants which was statistically superior to the other two categories. However, considering the seasonal variation in sulphur content, significantly high sulphur content of healthy plants was observed only during July-August. During February-March, the rhizosphere soils of yellowing affected plants showed significantly higher sulphur content compared to the other two categories.

The sulphur content during October-November was significantly lower in the rhizosphere soils of yellowing affected plants compared to other two categories. Thus, it can be seen from the table that sulphur content gave different pictures during different seasons in the rhizosphere soils of healthy, apparently healthy and yellowing

affected plants. Significantly low sulphur content was observed during July-August (22.85 mg Kg^{-1}).

4.2.1.10 Iron content

The iron content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 28. Iron content in the rhizosphere soils of healthy plants was significantly higher compared to apparently healthy plants. Iron content in the rhizosphere soils of yellowing affected plants was statistically on par with apparently healthy plants.

Considering season, the highest (32.81 mg kg^{-1}) iron content was observed during the October-November in the rhizosphere soils of black pepper, which was significantly higher than other two seasons.

During October-November and July-August iron content did not show significant difference in the rhizosphere soils of yellowing affected, apparently healthy and healthy plants. However, during February-March, iron content in the rhizosphere soils of healthy plants was significantly higher (35.44 mg kg^{-1}) compared to apparently healthy (24.71 mg kg^{-1}) and yellowing affected plants (26.71 mg kg^{-1}).

4.2.1.11 Manganese

The manganese content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 29. There was no significant difference in the manganese content in the rhizosphere soils of yellowing affected, apparently healthy and healthy plants. As can be seen from the table, seasonal mean manganese content was significantly higher during October-November (74.53 mg kg^{-1}) compared with February-March and July-August.

Table 27. Content of sulphur (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	24.23	18.39	**21.31**	27.04	27.46	**27.25**	32.58	29.96	**31.27**
A. H	22.18	19.45	**20.81**	33.15	27.00	**30.08**	31.07	27.82	**29.45**
Healthy	26.00	26.89	**26.45**	36.50	26.54	**31.52**	30.90	29.04	**29.97**
Year mean	**24.14**	**21.58**		**32.23**	**27.00**		**31.52**	**28.94**	
Seasonal mean	**22.86**			**29.62**			**30.23**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	27.95	25.27	**26.61**
Apparently healthy plants	28.80	24.75	**26.77**
Healthy plants	31.13	27.49	**29.31**
Category /Year mean	**29.29**	**25.83**	

	CD (0.05)		CD (0.05)
Season- (A)	**1.55**	Season x Year (A x B)	**2.20**
Year – (B)	**1.27**	Season x Category (A x C)	**2.69**
Category – (C)	**1.55**	Year x Category (B x C)	**2.20**
		Season x Year x Category (A x B x C)	**3.81**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 28. Content of iron (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	28.98	28.39	**28.68**	28.61	32.47	**30.54**	20.45	32.98	**26.71**
A. H	20.72	29.02	**24.87**	34.77	37.25	**36.01**	21.33	28.04	**24.68**
Healthy	28.97	28.33	**28.65**	29.55	34.21	**31.88**	35.50	35.40	**35.45**
Year mean	**26.22**	**28.58**		**30.98**	**34.64**		**25.76**	**32.14**	
Seasonal mean	**27.40**			**32.81**			**28.95**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	26.00	31.27	**28.64**
Apparently healthy plants	25.60	31.43	**28.52**
Healthy plants	31.34	32.64	**31.99**
Category /Year mean	**27.65**	**31.78**	

	CD (0.05)		CD (0.05)
Season- (A)	3.394	Season x Year (A x B)	**4.80**
Year – (B)	2.771	Season x Category (A x C)	**5.87**
Category – (C)	3.394	Year x Category (B x C)	**4.80**
		Season x Year x Category (A x B x C)	**8.31**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

During October-November manganese content in the rhizosphere soils of yellowing affected plants was significantly higher (82.55 mg kg^{-1}) compared to healthy plants. However, during other two seasons yellowing affected, apparently healthy and healthy were on par.

4.2.1.12 Zinc

The zinc content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 30. In the rhizosphere soils of healthy plant zinc content was significantly higher compared to apparently healthy and yellowing affected plants. The latter two were statistically on par.

There was no significant difference in rhizosphere soils of yellowing affected, apparently healthy and healthy plants in zinc content.

Zinc content in the rhizosphere soils of pepper plant during July-August and October-November were statistically on par. The highest (11.45 mg kg^{-1}) zinc content was noticed during February-March season, which was significantly higher than other two seasons. During October-November and July-August, zinc content did not show significant difference among the rhizosphere soils of yellowing affected, apparently healthy and healthy plants.

During February-March, healthy plants showed significantly higher (15.60 mg kg^{-1}) zinc content. However, yellowing affected and apparently healthy plants were statistically on par.

4.2.1.13 Copper

The copper content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 31.

Table 29. Content of manganese (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	40.27	80.30	**60.29**	83.84	81.25	**82.55**	57.65	46.68	**52.16**
A. H	38.54	70.39	**54.47**	79.54	67.87	**73.71**	57.35	51.41	**54.38**
Healthy	47.57	78.64	**63.11**	75.15	59.53	**67.34**	51.02	49.60	**50.31**
Year mean	**42.13**	**76.44**		**79.51**	**69.55**		**55.34**	**49.23**	
Seasonal mean	**59.29**			**74.53**			**52.29**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	60.58	69.41	**64.99**
Apparently healthy plants	58.47	63.22	**60.85**
Healthy plants	57.91	62.59	**60.25**
Category /Year mean	**58.99**	**65.07**	

	CD (0.05)		CD (0.05)
Season- (A)	**7.76**	Season x Year (A x B)	**10.98**
Year – (B)	**6.34**	Season x Category (A x C)	**13.45**
Category – (C)	**7.76**	Year x Category (B x C)	**10.98**
		Season x Year x Category (A x B x C)	**19.02**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 30. Content of zinc (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	6.49	4.57	**5.53**	4.43	8.09	**6.26**	8.50	9.14	**8.82**
A. H	4.99	4.17	**4.58**	4.18	6.69	**5.43**	7.29	12.57	**9.93**
Healthy	5.94	5.85	**5.90**	5.45	5.79	**5.62**	22.38	8.83	**15.61**
Year mean	**5.80**	**4.86**		**4.68**	**6.86**		**12.72**	**10.18**	
Seasonal mean	**5.33**			**5.77**			**11.45**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	6.47	7.26	**6.86**
Apparently healthy plants	5.48	7.81	**6.64**
Healthy plants	11.25	6.82	**9.04**
Category /Year mean	**7.73**	**7.30**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.96**	Season x Year (A x B)	**1.36**
Year – (B)	**0.78**	Season x Category (A x C)	**1.67**
Category – (C)	**0.96**	Year x Category (B x C)	**1.36**
		Season x Year x Category (A x B x C)	**2.36**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Copper content in the rhizosphere soils of yellowing affected, apparently healthy and healthy plants were statistically on par. Considering seasons, copper content was highest (21.01 mg kg^{-1}) during February-March which was significantly higher than other two seasons. Other two seasons were on par.

There was no significant difference in the copper content of yellowing affected, apparently healthy and healthy plants during October-November and July-August. During February-March, the rhizosphere soils of apparently healthy plants showed significantly lower (18.62 mg kg^{-1}) copper content compared to yellowing affected plants. Copper content in the rhizosphere soils of healthy plants were statistically on par with apparently healthy and yellowing affected plants.

4.2.1.14 Boron

The mean value of boron content was significantly higher (0.30 mg kg^{-1}**)** in rhizosphere soils from apparently healthy plants compared to yellowing affected and healthy plants (table 32).

During, October-November boron content was statistically on par in the three categories of the plant. During, February-March in rhizosphere soils from apparently healthy plants showed significantly high (0.40 mg kg^{-1}**)** boron content and other two categories were on par. During, July-August the rhizosphere soils of yellowing affected plants showed significantly lower (0.08 mg kg^{-1}) boron content. Apparently healthy and healthy were on par. Seasonal variation for boron content was observed with July-August showing significantly lowest boron content (0.18 mg kg^{-1}) compared to other two seasons.

4.2.1.15 Aluminium

Aluminium content in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants are presented in table 33. As can be seen from the table, aluminium content in the rhizosphere soils of healthy plant (4.53 mg

kg^{-1}) was significantly higher compared to yellowing affected and apparently healthy plants.

Considering the seasonal variation, the highest aluminium content (4.32 mg kg^{-1}) was observed during the July-August season, which was significantly higher than other two seasons. Aluminium content was lowest (2.30 mg kg^{-1}) during February-March, which was significantly lower than October-November (3.03 mg kg^{-1}) and July-August.

During February-March aluminium content did not show significant difference among apparently healthy and healthy plants. However, there was significant difference between yellowing affected and apparently healthy plants.

During July-August, aluminium content in the rhizosphere soils of healthy plants was significantly higher (7.41 mg kg^{-1}). Whereas yellowing affected plants showed significantly lowest aluminium content (2.22 mg kg^{-1}). When compared to apparently healthy (3.34 mg kg^{-1}) both were statistically on par. During October-November, the rhizosphere soils of apparently healthy plants recorded lowest aluminium content (1.86 mg kg^{-1}). Whereas healthy plants and yellowing affected plants were statistically on par.

Table 31. Content of copper (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	12.68	8.37	**10.53**	8.53	15.42	**11.97**	30.03	18.63	**24.33**
A. H	15.55	6.71	**11.13**	6.15	11.82	**8.98**	19.30	17.94	**18.62**
Healthy	15.56	14.15	**14.86**	12.53	9.93	**11.23**	20.04	20.13	**20.08**
Year mean	**14.60**	**9.74**		**9.07**	**12.39**		**23.13**	**18.90**	
Seasonal mean	**12.17**			**10.73**			**21.01**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	17.08	14.13	**15.60**
Apparently healthy plants	13.66	12.15	**12.91**
Healthy plants	16.04	14.73	**15.39**
Category /Year mean	**15.59**	**13.67**	

	CD (0.05)		CD (0.05)
Season- (A)	**3.07**	Season x Year (A x B)	**4.34**
Year – (B)	**2.50**	Season x Category (A x C)	**5.32**
Category – (C)	**3.07**	Year x Category (B x C)	**4.34**
		Season x Year x Category (A x B x C)	**7.52**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 32. Content of boron (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.08	0.08	**0.08**	0.32	0.21	**0.27**	0.17	0.38	**0.28**
A. H	0.26	0.18	**0.22**	0.42	0.16	**0.29**	0.35	0.44	**0.40**
Healthy	0.17	0.31	**0.24**	0.26	0.15	**0.21**	0.24	0.23	**0.24**
Year mean	**0.17**	**0.19**		**0.33**	**0.17**		**0.26**	**0.35**	
Seasonal mean	**0.18**			**0.25**			**0.30**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	0.19	0.22	**0.20**
Apparently healthy plants	0.34	0.26	**0.30**
Healthy plants	0.22	0.23	**0.22**
Category /Year mean	**0.25**	**0.23**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.05**	Season x Year (A x B)	**0.07**
Year – (B)	**0.04**	Season x Category (A x C)	**0.09**
Category – (C)	**0.05**	Year x Category (B x C)	**0.07**
		Season x Year x Category (A x B x C)	**0.13**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 33. Content of aluminium (mg kg^{-1}) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	1.26	3.19	**2.22**	4.31	2.53	**3.42**	1.53	0.99	**1.26**
A. H	2.89	3.80	**3.34**	2.31	1.42	**1.87**	4.12	2.47	**3.30**
Healthy	7.95	6.89	**7.42**	4.46	3.22	**3.84**	3.74	0.99	**2.36**
Year mean	**4.03**	**4.62**		**3.69**	**2.39**		**3.13**	**1.48**	
Seasonal mean	**4.33**			**3.04**			**2.31**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	2.36	2.23	**2.29**
Apparently healthy plants	3.10	2.56	**2.83**
Healthy plants	5.38	3.69	**4.53**
Category /Year mean	**3.61**	**2.83**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.72**	Season x Year (A x B)	**1.02**
Year – (B)	**0.59**	Season x Category (A x C)	**1.25**
Category – (C)	**0.72**	Year x Category (B x C)	**1.02**
		Season x Year x Category (A x B x C)	**1.77**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

4.3 EXPERIMENT III

Tissue analysis for nutrients

4.3.1 Nitrogen

Table 34 shows the plant nitrogen content in yellowing affected, apparently healthy and healthy black pepper plants. The nitrogen content was significantly highest (2.03 per cent) during February-March. The lowest (0.54 per cent) nitrogen content was recorded during July-August, which was significantly lower than February-March and October-November. During October-November and July-August, nitrogen content did not show significant difference among yellowing affected, apparently healthy and healthy plants. During February-March, healthy plant showed significantly high (3.00 per cent) nitrogen content when compared with apparently healthy and yellowing affected plants. Yellowing affected plants showed significantly lower nitrogen (0.92 per cent) content, when compared with apparently healthy plants. Treatment mean showed that the healthy plants contained significantly high nitrogen content when compared to apparently healthy and yellowing affected plants.

4.3.2 Phosphorus

Table 35 shows the plant phosphorus content in yellowing affected, apparently healthy and healthy black pepper plants. There was no significant variation in the phosphorus content among July-August, October- November and February-March. During July-August, phosphorus content did not show significant difference among yellowing affected, apparently healthy and healthy plants. However, healthy plants contained significantly higher phosphorus content when compared to apparently healthy and yellowing affected plants.

During October-November, healthy plants showed significantly higher (0.33 per cent) phosphorus content, compared to apparently healthy and yellowing affected

plants. However, yellowing affected plants showed significantly lower phosphorus (0.16 per cent) content when compared with apparently healthy and healthy plants. During February-March, healthy plant showed significantly higher phosphorus content (0.35 per cent) followed by (0.21 per cent) apparently healthy plants. However, yellowing affected plants showed significantly lower phosphorus content in plants (0.12 per cent) when compared with apparently healthy plants.

4.3.3 Potassium

Data analysis revealed that there was significant difference of plant potassium content in yellowing affected, apparently healthy and healthy black pepper plants (table 36). Healthy plants showed significantly high potassium content compared to other two categories and yellowing plants showed significantly lower potassium content. During July-August potassium content was significantly low compared to other two seasons.

During October-November, February-March and July-August potassium content showed significant difference among yellowing affected, apparently healthy and healthy plants. During October-November, healthy plant showed significantly highest potassium content in plant sample (2.27 per cent) followed by apparently healthy (1.88 per cent). The yellowing affected plants showed significantly lower potassium (1.42 per cent) content in the leaf samples.

During February-March, healthy plant showed significantly higher (2.31 per cent) potassium content in plant sample. The yellowing affected plants showed significantly lower potassium (1.59 per cent) content compared to healthy and was statistically on par with apparently healthy plants.

During July-August, healthy plant showed significantly highest (2.06 per cent) potassium content compared with apparently healthy and yellowing affected plants.

Table 34. Per cent nitrogen content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.45	0.43	**0.44**	1.11	1.01	**1.06**	1.07	1.46	**1.27**
A. H	0.43	0.52	**0.47**	1.30	1.32	**1.31**	1.58	2.07	**1.83**
Healthy	0.60	0.83	**0.72**	1.45	1.39	**1.42**	3.00	3.00	**3.00**
Year mean	**0.49**	**0.59**		**1.29**	**1.24**		**1.88**	**2.18**	
Seasonal mean	**0.54**			**1.26**			**2.03**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	0.88	0.97	**0.92**
Apparently healthy plants	1.10	1.30	**1.20**
Healthy plants	1.69	1.74	**1.71**
Category /Year mean	**1.22**	**1.34**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.18**	Season x Year (A x B)	**0.26**
Year – (B)	**0.15**	Season x Category (A x C)	**0.31**
Category – (C)	**0.18**	Year x Category (B x C)	**0.26**
		Season x Year x Category (A x B x C)	**0.45**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 35. Per cent phosphorous content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.15	0.15	**0.15**	0.15	0.17	**0.16**	0.14	0.12	**0.13**
A. H	0.22	0.21	**0.22**	0.27	0.25	**0.26**	0.20	0.24	**0.22**
Healthy	0.23	0.29	**0.26**	0.32	0.35	**0.34**	0.36	0.35	**0.36**
Year mean	**0.20**	**0.22**		**0.25**	**0.26**		**0.23**	**0.24**	
Seasonal mean	**NS**			**NS**			**NS**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	0.15	0.15	**0.15**
Apparently healthy plants	0.23	0.23	**0.23**
Healthy plants	0.31	0.33	**0.32**
Category /Year mean	**0.23**	**0.24**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.03**	Season x Year (A x B)	**0.04**
Year – (B)	**0.02**	Season x Category (A x C)	**0.05**
Category – (C)	**0.03**	Year x Category (B x C)	**0.04**
		Season x Year x Category (A x B x C)	**0.08**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 36. Per cent potassium content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	1.10	1.52	**1.31**	1.12	1.73	**1.42**	1.57	1.62	**1.59**
A. H	1.23	1.71	**1.47**	1.76	2.01	**1.89**	1.76	2.00	**1.88**
Healthy	2.07	2.07	**2.07**	2.02	2.54	**2.28**	1.92	2.71	**2.32**
Year mean	**1.47**	**1.77**		**1.63**	**2.09**		**1.75**	**2.11**	
Seasonal mean	**1.62**			**1.86**			**1.93**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	1.26	1.62	**1.44**
Apparently healthy plants	1.59	1.91	**1.75**
Healthy plants	2.00	2.44	**2.22**
Category /Year mean	**1.62**	**1.99**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.19**	Season x Year (A x B)	**0.27**
Year – (B)	**0.15**	Season x Category (A x C)	**0.33**
Category – (C)	**0.19**	Year x Category (B x C)	**0.27**
		Season x Year x Category (A x B x C)	**0.47**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

4.3.4 Calcium

Data analysis revealed that there was significant difference in calcium content in yellowing affected, apparently healthy and healthy black pepper plants (table 37).

The calcium content was significantly high in healthy plants compared to apparently healthy and yellowing affected plants. Calcium content was lowest in yellowing affected plants compared to other two categories.

The highest (3.40 per cent) calcium content was noticed during February-March season, which was significantly higher than other two seasons.

During all the seasons' calcium content was high in healthy plants compared to apparently healthy and yellowing affected plants.

4.3.5 Magnesium

As in the case of other nutrient elements, magnesium content also was significantly high in healthy plants compared to apparently healthy and yellowing affected plants. The latter two were statistically on par (table 38).

Seasonal variation in magnesium content showed that it was highest (1.58 per cent) during October-November. Magnesium content was on par during February-March and July-August.

Magnesium content was significantly higher (2.159 per cent) in healthy plants during October-November compared to apparently healthy and yellowing affected plants. During February-March and July-August there was no significant difference with respect to magnesium content in leaves of three categories of plants.

Table 37. Per cent calcium content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	1.73	1.43	**1.58**	1.38	1.42	**1.40**	1.53	1.35	**1.44**
A. H	3.08	1.85	**2.46**	1.60	1.76	**1.68**	4.24	3.33	**3.79**
Healthy	5.43	3.22	**4.33**	2.26	3.30	**2.78**	5.22	4.78	**5.00**
Year mean	**3.41**	**2.17**		**1.75**	**2.16**		**3.66**	**3.15**	
Seasonal mean	**2.79**			**1.95**			**3.41**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	1.55	1.40	**1.47**
Apparently healthy plants	2.97	2.31	**2.64**
Healthy plants	4.31	3.76	**4.04**
Category /Year mean	**2.94**	**2.49**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.25**	Season x Year (A x B)	**0.36**
Year – (B)	**0.21**	Season x Category (A x C)	**0.44**
Category – (C)	**0.25**	Year x Category (B x C)	**0.36**
		Season x Year x Category (A x B x C)	**0.62**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 38. Per cent magnesium content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.87	1.11	**0.99**	1.06	1.19	**1.13**	0.80	0.74	**0.77**
A. H	0.89	0.93	**0.91**	1.52	1.43	**1.47**	1.06	0.93	**0.99**
Healthy	1.29	1.49	**1.39**	2.39	1.93	**2.16**	1.10	1.14	**1.12**
Year mean	**1.01**	**1.18**		**1.65**	**1.52**		**0.98**	**0.93**	
Seasonal mean	**1.10**			**1.59**			**0.96**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	0.91	1.02	**0.96**
Apparently healthy plants	1.15	1.10	**1.12**
Healthy plants	1.59	1.52	**1.55**
Category /Year mean	**1.22**	**1.21**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.28**	Season x Year (A x B)	**0.39**
Year – (B)	**0.22**	Season x Category (A x C)	**0.48**
Category – (C)	**0.28**	Year x Category (B x C)	**0.39**
		Season x Year x Category (A x B x C)	**0.68**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

4.3.6 Sulphur

As can be seen from table 39 sulphur content was significantly high in healthy plants and significantly lowest in yellowing affected plants. Same trend was noticed during October-November and July-August. Seasonal variation in sulphur content showed that during July- August, sulphur content was significantly high compared to other two seasons and was lowest during February-March.

4.3.7 Iron

Data analysis revealed that there was significant difference in iron content of yellowing affected, apparently healthy and healthy black pepper plants.

The treatment means showed that the iron content was significantly highest in healthy plants when compared with apparently healthy and yellowing affected plants. (table 40). Yellowing affected plants were low in iron content compared to other categories.

During, October-November significantly high iron content (916.0 mg kg^{-1}) was observed, when compared to other seasons. Whereas February- March recorded significantly low iron content (612.21 mg kg^{-1}), when compared with July-August season.

During October-November, healthy plants showed significantly high iron content (1200.45 mg kg^{-1}). Yellowing affected plants showed significantly low iron (660.20 mg kg^{-1}) content in the leaf samples when compared with apparently healthy and healthy plant sample.

During July-August yellowing affected plants showed significantly low iron (641.73 mg kg^{-1}) content in the leaf samples. Other two categories were on par.

4.3.8 Manganese

The treatment means showed that manganese content was significantly higher in healthy plants when compared to apparently healthy and yellowing affected plants (table 41). Yellowing affected plants showed significantly low manganese content.

The manganese content was highest during February-March (512.36 mg kg^{-1}). Other two seasons were on par.

During February-March healthy plant showed significantly high (783.40 mg kg^{-1}) manganese content in plant sample, followed by apparently healthy. However, yellowing affected plants showed significantly lower manganese (287.76 mg kg^{-1}) content in the leaf samples. During October-November and July-August also same trend was noticed.

4.3.9 Zinc

Perusal of the data revealed that there was a significant difference in the zinc content of the plant sample (table 42). As can be seen from the table, treatment mean showed that zinc content in healthy plants was significantly higher when compared to apparently healthy and yellowing affected plants. Yellowing affected plants showed significantly low zinc content.

Among seasons zinc content was highest during February-March and lowest during October-November.

During February-March, healthy plants showed significantly high (71.95 mg kg^{-1}) zinc content. Yellowing affected plants showed significantly lower zinc content (37.58 mg kg^{-1}) in plants when compared to healthy and apparently healthy plants.

During July-August and October-November zinc content was lowest in yellowing affected plants compared to other two categories.

Table 39. Per cent sulphur content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.09	0.23	**0.16**	0.05	0.07	**0.06**	0.04	0.06	**0.05**
A. H	0.13	0.26	**0.20**	0.08	0.11	**0.09**	0.07	0.14	**0.11**
Healthy	0.14	0.37	**0.26**	0.16	0.27	**0.22**	0.12	0.15	**0.13**
Year mean	**0.12**	**0.29**		**0.10**	**0.15**		**0.08**	**0.12**	
Seasonal mean	**0.21**			**0.12**			**0.10**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	0.06	0.12	**0.09**
Apparently healthy plants	0.09	0.17	**0.13**
Healthy plants	0.14	0.26	**0.20**
Category /Year mean	**0.10**	**0.18**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.02**	Season x Year (A x B)	**0.03**
Year – (B)	**0.01**	Season x Category (A x C)	**0.04**
Category – (C)	**0.02**	Year x Category (B x C)	**0.03**
		Season x Year x Category (A x B x C)	**0.05**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 40. Per cent iron content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	625.57	657.90	**641.73**	607.90	712.50	**660.20**	504.63	562.27	**533.45**
A. H	820.53	760.80	**790.67**	719.50	1055.47	**887.49**	642.43	597.43	**619.93**
Healthy	731.13	831.70	**781.42**	721.70	1679.20	**1200.45**	682.60	683.93	**683.27**
Year mean	**725.74**	**750.13**		**683.03**	**1149.05**		**609.89**	**614.54**	
Seasonal mean			**737.94**			**916.04**			**612.22**

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	579.37	644.22	**611.79**
Apparently healthy plants	727.49	804.57	**766.03**
Healthy plants	711.81	1064.95	**888.38**
Category /Year mean	**672.89**	**837.91**	

	CD (0.05)		CD (0.05)
Season- (A)	**59.65**	Season x Year (A x B)	**84.36**
Year – (B)	**48.70**	Season x Category (A x C)	**103.32**
Category – (C)	**59.65**	Year x Category (B x C)	**84.36**
		Season x Year x Category (A x B x C)	**146.12**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 41. Per cent manganese content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	350.00	235.23	**292.62**	260.27	228.43	**244.35**	292.90	264.63	**278.77**
A. H	376.37	361.90	**369.13**	345.23	397.83	**371.53**	426.40	523.47	**474.93**
Healthy	480.80	398.90	**439.85**	371.07	484.17	**427.62**	751.83	814.97	**783.40**
Year mean	**402.39**	**332.01**		**325.52**	**370.14**		**490.38**	**534.36**	
Seasonal mean	**367.20**			**347.83**			**512.37**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	301.06	242.77	**271.91**
Apparently healthy plants	382.67	427.73	**405.20**
Healthy plants	534.57	566.01	**550.29**
Category /Year mean	**406.10**	**412.17**	

	CD (0.05)		CD (0.05)
Season- (A)	**42.31**	Season x Year (A x B)	**59.84**
Year – (B)	**34.55**	Season x Category (A x C)	**73.29**
Category – (C)	**42.31**	Year x Category (B x C)	**59.84**
		Season x Year x Category (A x B x C)	**103.65**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 42. Per cent Zinc content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	32.30	51.87	**42.08**	32.80	22.01	**27.41**	26.57	48.60	**37.59**
A. H	40.07	60.27	**50.17**	38.33	27.97	**33.15**	28.17	69.80	**48.98**
Healthy	41.73	65.17	**53.45**	40.40	33.00	**36.70**	44.03	99.87	**71.95**
Year mean	**38.03**	**59.10**		**37.18**	**27.66**		**32.92**	**72.76**	
Seasonal mean	**48.57**			**32.42**			**52.84**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	30.56	40.83	**35.69**
Apparently healthy plants	35.52	52.68	**44.10**
Healthy plants	42.06	66.01	**54.03**
Category /Year mean	**36.05**	**53.17**	

	CD (0.05)		CD (0.05)
Season- (A)	**3.34**	Season x Year (A x B)	**4.73**
Year – (B)	**2.73**	Season x Category (A x C)	**5.79**
Category – (C)	**3.34**	Year x Category (B x C)	**4.73**
		Season x Year x Category (A x B x C)	**8.20**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

4.3.10 Copper

Table 43 shows the copper content in the plant samples of yellowing affected, apparently healthy and healthy black pepper plants. The treatment mean showed that the copper content in healthy plants was significantly higher when compared to apparently healthy and yellowing affected plants. Copper content was lowest in yellowing affected plants.

The copper content was significantly highest (24.17 mg kg^{-1}) during February-March season followed by October-November (15.20 mg kg^{-1}). The lowest copper content was noticed during July-August (9.61 mg kg^{-1}).

4.3.11 Boron

The treatment means showed that the healthy plants contained significantly higher boron content when compared to apparently healthy and yellowing affected plants (table 44). The highest (51.99 mg kg^{-1}) boron content was noticed during February-March season. This was significantly higher than other two seasons which were on par.

During February-March and July-August, boron content was significantly high in healthy plants compared to other two categories.

4.3.12 Aluminum

As can be seen from the table 45, aluminium content was significantly high in healthy plants when compared with apparently healthy and yellowing affected plants. Aluminium content was lowest (375.68 mg kg^{-1}) during February-March, which was significantly lower than other two seasons which were on par.

During July-August and February-March, healthy plants showed significantly highest aluminium content in plant sample. However, yellowing affected plants showed significantly lower aluminium content in the leaf samples.

Table 43. Per cent copper content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	7.37	7.43	**7.40**	3.63	19.55	**11.59**	10.93	16.64	**13.79**
A. H	8.40	12.30	**10.35**	7.13	23.43	**15.28**	13.37	29.80	**21.58**
Healthy	12.03	10.13	**11.08**	7.23	30.24	**18.74**	34.10	40.23	**37.17**
Year mean	**9.27**	**9.96**		**6.00**	**24.41**		**19.47**	**28.89**	
Seasonal mean	**9.61**			**15.20**			**24.18**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	7.31	14.54	**10.93**
Apparently healthy plants	9.63	21.84	**15.74**
Healthy plants	17.79	26.87	**22.33**
Category /Year mean	**11.58**	**21.08**	

	CD (0.05)		CD (0.05)
Season- (A)	**2.80**	Season x Year (A x B)	**3.97**
Year – (B)	**2.29**	Season x Category (A x C)	**4.86**
Category – (C)	**2.80**	Year x Category (B x C)	**3.97**
		Season x Year x Category (A x B x C)	**6.87**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 44. Per cent boron content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	28.37	29.57	**28.97**	26.00	31.40	**28.70**	35.70	36.50	**36.10**
A. H	29.26	41.07	**35.16**	31.80	35.57	**33.68**	46.03	45.80	**45.92**
Healthy	49.63	42.40	**46.02**	32.17	42.73	**37.45**	82.80	65.13	**73.97**
Year mean	**35.75**	**37.68**		**29.99**	**36.57**		**54.84**	**49.14**	
Seasonal mean	**36.72**			**33.28**			**51.99**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	30.02	32.49	**31.26**
Apparently healthy plants	35.70	40.81	**38.25**
Healthy plants	54.87	50.09	**52.48**
Category /Year mean	**40.20**	**41.13**	

	CD (0.05)		CD (0.05)
Season- (A)	**4.41**	Season x Year (A x B)	**6.24**
Year – (B)	**3.60**	Season x Category (A x C)	**7.65**
Category – (C)	**4.41**	Year x Category (B x C)	**6.24**
		Season x Year x Category (A x B x C)	**10.82**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 45. Per cent aluminium content in the leaves of yellowing affected, apparently healthy and healthy plants of selected black pepper fields

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	460.73	295.50	**378.12**	473.47	528.87	**501.17**	233.70	290.47	**262.08**
A. H	614.40	551.05	**582.73**	582.77	734.33	**658.55**	352.13	385.27	**368.70**
Healthy	815.73	612.47	**714.10**	622.83	647.80	**635.32**	505.83	486.70	**496.27**
Year mean	**630.29**	**486.34**		**559.69**	**637.00**		**363.89**	**387.48**	
Seasonal mean	**558.31**			**598.34**			**375.68**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	389.30	371.61	**380.46**
Apparently healthy plants	516.43	556.88	**536.66**
Healthy plants	648.13	582.32	**615.23**
Category /Year mean	**517.96**	**503.61**	

	CD (0.05)		CD (0.05)
Season- (A)	**54.67**	Season x Year (A x B)	**77.31**
Year – (B)	**44.63**	Season x Category (A x C)	**94.69**
Category – (C)	**54.67**	Year x Category (B x C)	**77.31**
		Season x Year x Category (A x B x C)	**133.91**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H

During October-November, yellowing affected plants showed significantly low aluminum content (501.167 mg kg^{-1}). Healthy and apparently healthy plants were on par.

4.4 EXPERIMENT IV

Study on association of plant pathogenic organisms with yellowing

4.4.1 Per cent disease incidence

During July-August (2018), highest per cent incidence of yellowing was recorded in location 2 (26 per cent) followed by location 3 (16.46 per cent). The lowest incidence was recorded in location 6 (2.06 per cent). The same trend was observed during 2019 (table 46).

During October-November (2018) also, highest per cent incidence of yellowing was recorded in location 2 (46 per cent) followed by location 3 (31.5 per cent). The lowest was recorded in location 6 (8.24 per cent). The same trend was observed during 2019.

During February-March (2018), highest per cent incidence of yellowing was recorded highest in location 2 (36 per cent) followed by location 3 (23 per cent). The lowest was recorded in location-6 (5.15 per cent). The same trend was observed during 2019.

Mean value showed that during 2017 per cent disease incidence was significantly high during October-November and lowest during July-August in location 5 and location 6. During 2018, the differences were not significant.

4.4.2 Per cent disease severity

Observations were reported on yellowing affected, apparently healthy and healthy plants during July-August, October-November and February-March in 2017-18 (table 47).

During July-August in both the years yellowing affected plants showed significantly highest disease severity (44.59 and 58.36 per cent) in location 2. The lowest disease severity was recorded in healthy plants (0.00 per cent) in location 6.

During October-November (2017 and 2018) yellowing affected plants showed significantly highest disease severity (46.66; 45.18 per cent) in location 2. The lowest disease severity was recorded in healthy plants (0.82; 0.11 per cent) in location 6.

During February-March (2017 and 2018) significantly highest disease severity (47.55; 74.96 per cent) was observed in location 2. The lowest disease severity was recorded (0.32; 0.22 per cent) in location 6.

4.4.3 Soil micro flora from suppressive soil

4.4.3.1 Fungal population

Considerable variation in the population of fungi was noticed during July-August, October-November and February-March during two consecutive years. Table 48 shows population of fungi in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants.

The mean value of fungal population was highest in rhizosphere soils of healthy plants. It was significantly superior to apparently healthy and yellowing affected plants, the latter two were statistically on par.

Comparing the seasons, over all fungal population was significantly highest during February-March, compared to July-August and October-November.

Fungal population in the rhizosphere soil was significantly low during July-August compared to other two seasons. During July-August, there was no significant difference in the fungal population in the rhizosphere soil of yellowing affected, apparently healthy and healthy plants. During October-November, healthy plants showed significantly high (20.76×10^{3} g^{-1}cfu) fungal population. Yellowing affected

plants showed significantly least population compared to healthy and apparently healthy plants.

During February-March, rhizosphere soils of yellowing affected plants showed significantly high fungal count (24.04 $\times 10^{3}$ g^{-1}cfu) when compared with apparently healthy plants (18.06 $\times 10^{3}$ g^{-1}cfu) whereas apparently healthy plants were on par with healthy plants.

4.4.3.2 Actinomycetes population

It was observed that (table 49) there was significant difference in the population of actinomycetes during July-August, October-November and February-March during the period of study.

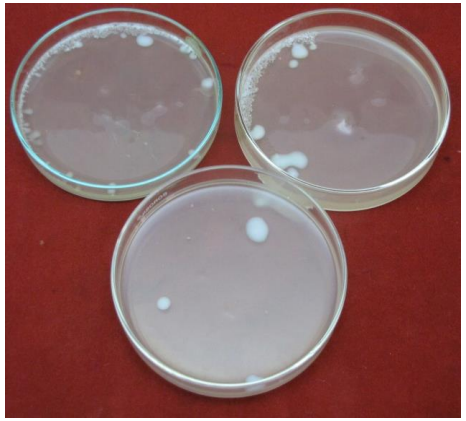
Healthy plants showed significantly low population count of (22.34 $\times 10^{5}$ g^{-1}cfu) actinomycetes in the rhizosphere soil, whereas apparently healthy and yellowing affected plants were statistically on par.

Actinomycetes population was significantly highest (34.73 $\times 10^{5}$ g^{-1}cfu) during the February-March season. The least count of actinomycetes population was noticed during July-August (18.48 $\times 10^{5}$ g^{-1}cfu) which was significantly lower compared to other two seasons.

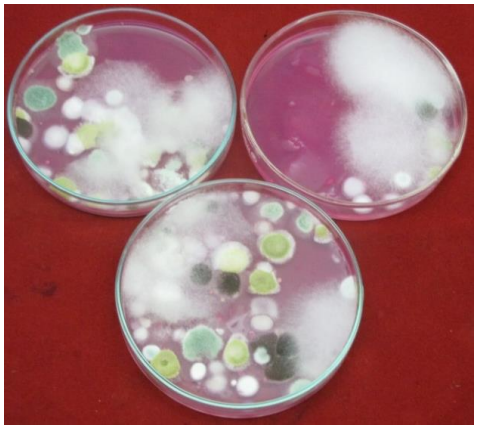
During July-August, yellowing affected plants showed significantly low population of actinomycetes (13.80 $\times 10^{5}$ g^{-1}cfu) in the rhizosphere soil compared to apparently healthy and healthy plants.

During October-November, yellowing affected plants showed significantly highest (28.51 $\times 10^{5}$ g^{-1}cfu) actinomycetes population. Healthy plants showed significantly least population compared to apparently healthy plants and yellowing affected plants, both were statistically on par.

Plate 8. Micro flora from rhizosphere soil of experimental plants



Actinomycetes



Fungi

Bacteria

Table 46. Per cent disease incidence (PDI) in yellowing during three seasons

Per cent disease incidence (PDI) 2018-19								
Locations	**July-August 2017**	**July-August 2018**	**October-November 2017**	**October-November 2018**	**February-March 2018**	**February-March 2019**	**Mean 2017**	**Mean 2018**
L-1	3.45	2.36	20.36	23.63	18.54	14.18	14.11 [bc]	13.39
L-2	26	18	46	54	36	26	36.00 [a]	32.66
L-3	16.46	14.43	31.5	30	23	18.5	23.65 [ab]	20.97
L-4	4.3	2.33	18	19.66	21.39	13	14.56 [bc]	11.66
L-5	5.11	4.22	8.66	10.66	11.33	8.66	8.36 [c]	7.84
L-6	2.06	0	8.24	13.4	5.15	2.06	5.15 [c]	5.15
CD (0.05)							**13.47**	**NS**

Locations-1, Locations-2, Locations-3, Locations-4, Locations-5, Locations-6. Per cent disease incidence (PDI)

Table 47. Seasonal variation in the per cent disease severity (PDS) in yellowing affected, apparently healthy and healthy black pepper plants

		July-August (2017)	July-August (2018)	October-November (2017)	October-November (2018)	February-March (2018)	February-March (2019)
Locations	**Treatments**	**PDS**	**PDS**	**PDS**	**PDS**	**PDS**	**PDS**
L-1	Y	17.22 ^{d}	12.58 ^{cd}	32.58 ^{d}	29.43 ^{bc}	26.88 ^{bcd}	35.40 ^{bc}
	A.H	1.32 ^{g}	6.66 ^{e}	**40.58 ^{abc}**	14.81 ^{e}	15.25 ^{ef}	41.48 ^{b}
L-2	Y	**44.59 ^{a}**	**58.36 ^{a}**	**46.66 ^{a}**	**45.18 ^{a}**	**47.55 ^{a}**	**74.96 ^{a}**
	A.H	5.32 ^{f}	**26.81 ^{b}**	20.03 ^{e}	**27.10 ^{c}**	**28.58 ^{bc}**	**39.48 ^{b}**
L-3	Y	40.29 ^{b}	8.88 ^{de}	41.62 ^{ab}	30.66 ^{bc}	32.88 ^{b}	26.51 ^{cd}
	A.H	8.14 ^{ef}	16.29 ^{c}	31.40 ^{d}	22.07 ^{d}	19.99 ^{de}	37.62 ^{bc}
L-4	Y	24.44 ^{c}	13.33 ^{cd}	36.88 ^{bcd}	27.25 ^{c}	46.21 ^{a}	17.33 ^{de}
	A.H	5.33 ^{f}	12.73 ^{cd}	17.47 ^{e}	18.66 ^{de}	11.70 ^{f}	18.36 ^{de}
L-5	Y	25.77 ^{c}	29.52 ^{b}	34.21 ^{cd}	43.99 ^{a}	25.18 ^{cd}	12.43 ^{e}
	A.H	**8.67 ^{e}**	16.14 ^{c}	35.55 ^{bcd}	31.99 ^{b}	27.69 ^{bc}	22.51 ^{de}
L-6	Healthy	**0.00 ^{g}**	**0.00 ^{f}**	**0.82 ^{f}**	**0.11 ^{f}**	**0.32 ^{g}**	**0.22 ^{f}**
CD (0.05)		**3.30**	**4.81**	**6.84**	**4.69**	**7.00**	**11.47**

Locations-1, Locations-2, Locations-3, Locations-4, Locations-5, Locations-6. Per cent disease severity (PDS)

Table 48. Fungal population in soils of yellowing affected, apparently healthy and healthy black pepper plants (×10 ^{3} g^{-1}cfu)

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	12.44	12.85	**12.64**	14.22	12.87	**13.54**	16.25	31.85	**24.05**
A.H	14.69	14.94	**14.81**	11.29	17.80	**14.55**	18.00	18.13	**18.07**
Healthy	13.98	12.33	**13.16**	21.91	19.62	**20.77**	23.29	21.56	**22.42**
Year mean	**13.70**	**13.37**		**15.81**	**16.76**		**19.18**	**23.84**	
Seasonal mean	**13.54**			**16.29**			**21.51**		

Category / Year (C)	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	14.30	19.19	**16.74**
Apparently healthy plants	14.66	16.96	**15.81**
Healthy plants	19.73	17.84	**18.78**
Category /Year mean	**16.23**	**17.99**	

	CD (0.05)		CD (0.05)
Season- (A)	**1.70**	Season x Year (A x B)	**2.41**
Year – (B)	**1.39**	Season x Category (A x C)	**2.95**
Category – (C)	**1.70**	Year x Category (B x C)	**2.41**
		Season x Year x Category (A x B x C)	**4.180**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 49. Actinomycetes population in soils of yellowing affected, apparently healthy and healthy black pepper plants (×10 ^{5} g^{-1}cfu)

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	12.36	15.25	**13.80**	23.82	33.20	**28.51**	36.27	44.38	**40.32**
A.H	19.96	21.33	**20.65**	22.40	26.60	**24.50**	39.22	35.93	**37.58**
Healthy	20.07	21.98	**21.02**	21.36	18.09	**19.72**	18.36	34.24	**26.30**
Year mean	17.46	19.52		22.53	25.96		31.28	38.19	
Seasonal mean	**18.49**			**24.24**			**34.73**		

Category / Year (C)	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	24.15	30.94	27.55
Apparently healthy plants	27.19	27.96	27.57
Healthy plants	19.93	24.77	22.35
Category /Year mean	23.76	27.89	

	CD (0.05)		CD (0.05)
Season- (A)	**2.24**	Season x Year (A x B)	**3.17**
Year – (B)	**1.83**	Season x Category (A x C)	**3.89**
Category – (C)	**2.24**	Year x Category (B x C)	**3.17**
		Season x Year x Category (A x B x C)	**5.50**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 50. Bacterial population in soils of yellowing affected, apparently healthy and healthy black pepper plants ($\times 10^{8}$ g^{-1}cfu)

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	13.16	15.38	**14.27**	45.31	48.84	**47.08**	13.36	17.18	**15.27**
A.H	16.18	23.80	**19.99**	23.16	40.53	**31.85**	16.58	11.47	**14.02**
Healthy	17.16	14.20	**15.68**	18.98	16.20	**17.59**	21.98	18.87	**20.42**
Year mean	15.50	17.79		29.15	35.19		17.30	15.84	
Seasonal mean	**16.64**			**32.17**			**16.57**		

Category / Year (C)	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	23.94	27.13	**25.53**
Apparently healthy plants	18.63	25.26	**21.95**
Healthy plants	19.37	16.42	**17.89**
Category /Year mean	20.64	22.94	

	CD (0.05)		CD (0.05)
Season- (A)	**2.33**	Season x Year (A x B)	3.30
Year – (B)	1.90	Season x Category (A x C)	**4.04**
Category – (C)	**2.33**	Year x Category (B x C)	3.30
		Season x Year x Category (A x B x C)	5.72

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

During February-March, healthy plants showed significantly lower population of (26.30 $\times 10^5$ g^{-1}cfu) actinomycetes in the rhizosphere soil compared to apparently healthy and yellowing affected plants.

4.4.3.3 Bacterial population

It was observed that (table 50) there was significant difference in the population of bacteria in July-August, October-November and February-March during two consecutive years.

Category mean of yellowing affected plants showed significantly highest population count of bacteria (25.53 $\times 10^8$ g^{-1}cfu) followed by apparently healthy plants, whereas healthy plants showed significantly lowest count of bacterial population.

Bacterial population was significantly highest (32.17 $\times 10^8$ g^{-1}cfu) during October-November. The least count of bacterial population was noticed during July-August (16.64 $\times 10^8$ g^{-1}cfu) which was statistically on par with February-March (16.57 $\times 10^5$ g^{-1}cfu).

During July-August, October- November and February-March there was significant difference in the bacterial population among yellowing affected, apparently healthy and healthy plants.

During July-August apparently healthy plants showed significantly highest bacterial count (19.98 $\times 10^8$ g^{-1}cfu). Yellowing affected plants showed significantly lowest count (14.26 $\times 10^8$ g^{-1}cfu) in the rhizosphere soil. Apparently healthy plants were statistically on par with healthy plants.

During October-November, yellowing affected plants showed significantly highest (47.07 $\times 10^8$ g^{-1}cfu) bacterial population. Healthy plants showed significantly

least population (17.58 ×10 ^{8} g^{-1}cfu) compared to apparently healthy plants and yellowing affected plants, which were statistically on par.

During February-March, healthy plants showed significantly highest population (20.42 ×10 ^{8} g^{-1}cfu) count. Apparently healthy plants showed significantly lower population count (14.02 ×10 ^{8} g^{-1}cfu) of bacteria in the rhizosphere soil. Healthy plants where was statistically on par with apparently healthy plants.

4.4.4 Plant pathogenic micro flora

Any plant pathogenic organisms causing yellowing in black pepper could not be isolated either from roots or rhizosphere soil (*Fusarium, Rhizactonia* and *Phytopthora* are reported cause yellowing of black pepper).

4.5 EXPERIMENT-V

Analysis of rhizosphere soil and root samples for nematode

4.5.1 Nematode population in 250 cm^3 soil (number/250 cm^3 soil)

Nematode population was observed during all the three seasons under study. During October-November nematode population in the rhizosphere soils of healthy plants was significantly lower compared to apparently healthy and yellowing affected plants (table 51). Nematode population was significantly low in healthy plants during July-August. Whereas yellowing affected and apparently healthy plants were statistically on par.

During July-August the nematode population in the rhizosphere was significantly lowest compared to October-November and February-March.

During October-November the nematode population in rhizosphere soils was significantly highest compared to February-March and July-August.

Plate 9. Symptoms yellowing during various seasons of the year

July-August

October-November

February-March

The rhizosphere soils of yellowing affected plants showed significantly higher nematode population, whereas healthy plants recorded significantly lowest nematode population when compared to apparently healthy and yellowing affected plants.

4.5.2 Constitution (genera) of plant parasitic nematodes from the surveyed area

Different species of nematodes were obtained from surveyed area like *Meloidogyne incognita, Radopholus similis, Pratylenchus, Helicotylenchus* sp and *Dorylamid sp*.

4.5.2.1 Meloidogyne incognita in soils

Table 52 shows the *M. incognita* population in the rhizosphere soils of yellowing affected, apparently healthy and healthy plants. As can be seen from the table, population was significantly low in the rhizosphere soils of healthy plants and was on par in yellowing affected plants and apparently healthy plants.

The population of *Meloidogyne incognita* was significantly lowest during rainy season (July-August). The population was significantly highest during October-November.

The population of *M. incognita* was significantly lowest in the rhizosphere soils of healthy plants during July-August, October-November and February-March. However, apparently healthy and yellowing affected plants were statistically on par during all the three seasons.

4.5.2.2 Radopholus similis population in soil

The population of *Radopholus similis* was significantly lowest in the rhizosphere soils of healthy plants when compared with yellowing affected and apparently healthy (table 53).

However, yellowing affected plants showed significantly highest *Radopholus similis* population when compared with apparently healthy plants.

During February-March, in the rhizosphere soils of yellowing affected plants significantly higher number of *Radopholus similis* population was observed compared to apparently healthy and healthy plants. During, October-November yellowing affected plants were statistically on par with apparently healthy plants. During July-August, all the three categories were on par for *Radopholus similis.*

4.5.2.3 Population of Pratylenchus sp. in soil

Pratylenchus population was absent in the rhizosphere soils of healthy plants during the entire course of observation (table 54). Treatment mean showed that the population was significantly highest in yellowing affected plants when compared with apparently healthy.

Pratylenchus population in the rhizosphere soil was statistically on par in yellowing affected and healthy plants, during July-August. In apparently healthy plants the population was significantly high.

During October-November and February-March also the population of *Pratylenchus* was zero in the rhizosphere soils of healthy plants. It was significantly highest in the rhizosphere soils of yellowing affected plants during two seasons.

4.5.2.4 Helicotylenchus sp. population in soils

The population of *Helicotylenchus* sp was significantly lowest in healthy plants, and was significantly highest in the rhizosphere soils of apparently healthy plants (table 55).

Table 51. Total nematode population (no/250 cc soil) in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	174.60 (12.90)	146.60 (12.02)	**160.60 (12.46)**	1281.20 (34.80)	926.00 (28.31)	**1103.60 (31.55)**	861.20 (26.61)	555.00 (21.79)	**708.10 (24.20)**
A. H	264.60 (15.61)	116.20 (10.02)	**190.40 (12.81)**	863.40 (27.74)	784.20 (26.38)	**823.80 (27.06)**	639.80 (24.59)	352.80 (18.42)	**496.30 (21.50)**
Healthy	0.80 (1.04)	0.80 (1.04)	**0.80 (1.04)**	7.80 (2.48)	7.00 (2.68)	**7.40 (2.58)**	2.40](1.52)	12.80 (3.53)	**7.60 (2.53)**
Year mean	**146.66** (9.85)	**87.86** (7.69)		**717.46** (21.67)	**572.40** (19.12)		**501.13** (17.57)	**306.86** (14.58)	
Seasonal mean	**117.2 (8.77)**			**644.9 (20.40)**			**404.00 (16.08)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	772.33 (24.77)	542.53 (20.70)	**657.43 (22.74)**
Apparently healthy plants	589.26 (22.64)	417.73 (18.27)	**503.50 (20.46)**
Healthy plants	3.66 (1.63)	6.86 (2.42)	**5.26 (2.05)**
Category /Year mean	**455.08 (16.36)**	**322.37 (13.80)**	

	CD (0.05)		CD (0.05)
Season- (A)	**3.58**	Season x Year (A x B)	**5.06**
Year – (B)	**2.92**	Season x Category (A x C)	**6.20**
Category – (C)	**3.58**	Year x Category (B x C)	**5.06**
		Season x Year x Category (A x B x C)	**8.77**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 52. *Meloidogyne incognita* population in the rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	35.40 (5.65)	35.00 (5.78)	**35.20 (5.71)**	88.60 (9.40)	77.40 (8.77)	**83.00 (9.08)**	101.00 (10.04)	103.40 (10.15)	**102.20 (10.10)**
A. H	11.60 (3.44)	33.00 (5.56)	**22.30 (4.50)**	73.20 (8.58)	104.40 (9.83)	**88.80 (9.21)**	99.80 (9.97)	83.40 (9.11)	**91.60 (9.54)**
Healthy	0.00 (0.71)	0.80 (1.04)	**0.40 (0.87)**	2.40 (1.67)	2.40 (1.59)	**2.40 (1.63)**	0.00 (0.71)	0.40 (0.88)	**0.20 (0.79)**
Year mean	**15.66 (3.27)**	**22.93 (4.12)**		**54.73 (6.55)**	**61.40 (6.73)**		**66.93(6.91)**	**62.40 (6.71)**	
Seasonal mean	**19.30 (3.70)**			**58.06 (6.64)**			**64.66 (6.81)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	75.00 (8.36)	71.93 (8.23)	**73.46 (8.30)**
Apparently healthy plants	61.53 (7.33)	73.60 (8.17)	**67.56 (7.75)**
Healthy plants	0.80 (1.03)	1.20 (1.17)	1.00 (1.10)
Category /Year mean	**45.77 (5.57)**	**48.91 (5.86)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.64**	Season x Year (A x B)	**0.91**
Year – (B)	**0.53**	Season x Category (A x C)	**1.12**
Category – (C)	**0.64**	Year x Category (B x C)	**0.91**
		Season x Year x Category (A x B x C)	**1.59**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H).
Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 53. Population of *Radopholus similis* in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	15.80 (3.90)	16.60 (3.90)	**16.20 (3.90)**	156.40 (12.51)	91.20 (9.32)	**123.80 (10.91)**	156.40 (12.44)	115.80 (10.75)	**136.10 (11.59)**
A. H	7.00 (2.74)	6.20 (2.47)	**6.60 (2.59)**	141.40 (11.83)	79.80 (8.81)	**110.60 (10.32)**	88.60 (9.36)	73.20 (8.43)	**80.90 (8.90)**
Healthy	0.00 (0.71)	0.00 (0.71)	**0.00 (0.70)**	3.40 (1.79)	2.60 (1.57)	**3.00 (1.68)**	0.00 (0.71)	0.20 (0.81)	**0.10 (0.76)**
Year mean	**7.60 (2.44)**	**7.60 (2.36)**		**100.40 (8.71)**	**57.86 (6.57)**		**81.66 (7.50)**	**63.06 (6.66)**	
Seasonal mean	**7.60 (2.40)**			**79.13 (7.64)**			**72.36 (7.08)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	109.53 (9.62)	74.53 (7.99)	**92.03 (8.80)**
Apparently healthy plants	79.00 (7.97)	53.06 (6.57)	**66.03 (7.27)**
Healthy plants	1.13 (1.07)	0.93 (1.03)	**1.03 (1.05)**
Category /Year mean	**63.22 (6.22)**	**42.84 (5.19)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.60**	Season x Year (A x B)	**0.85**
Year – (B)	**0.49**	Season x Category (A x C)	**1.05**
Category – (C)	**0.60**	Year x Category (B x C)	**0.85**
		Season x Year x Category (A x B x C)	**1.48**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 54. *Pratylenchus* sp. population in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.80 (1.04)	1.00 (1.14)	**0.90 (1.09)**	11.40 (3.42)	6.80 (2.63)	**9.10 (3.03)**	10.80 (3.34)	7.80 (2.81)	**9.30 (3.07)**
A. H	3.00 (1.79)	5.20 (2.15)	**4.10 (1.97)**	10.20 (3.24)	2.60 (1.62)	**6.40 (2.43)**	5.00 (2.32)	3.40 (1.85)	**4.20 (2.09)**
Healthy	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Year mean	**1.26 (1.18)**	**2.06 (1.33)**		**7.20 (2.46)**	**3.13 (1.65)**		**5.26 (2.12)**	**3.73 (1.79)**	
Seasonal mean	**1.66 (1.26)**			**5.16 (2.05)**			**4.50 (1.96)**		

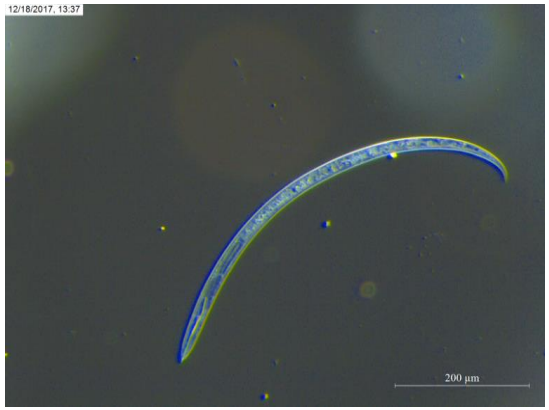
Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	7.667 (2.60)	5.20 (2.19)	**6.43 (2.40)**
Apparently healthy plants	6.06 (2.45)	3.73(1.87)	**4.90 (2.16)**
Healthy plants	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Category /Year mean	**4.57 (1.92)**	**2.97 (1.59)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.26**	Season x Year (A x B)	**0.37**
Year – (B)	**0.21**	Season x Category (A x C)	**0.45**
Category – (C)	**0.26**	Year x Category (B x C)	**0.37**
		Season x Year x Category (A x B x C)	**0.64**

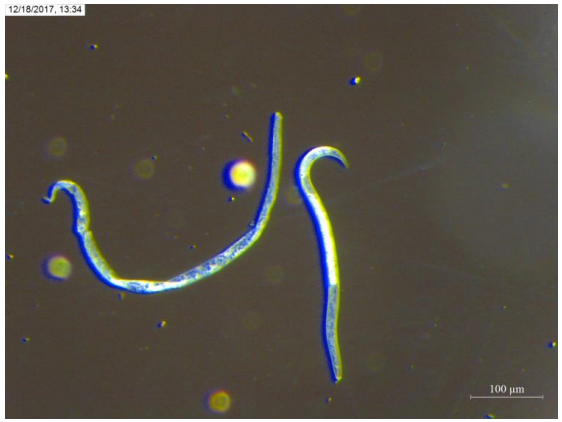
Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

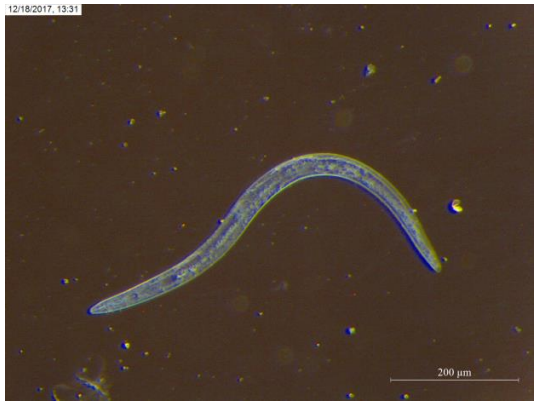
Plate. 10. Nematode spp. in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants



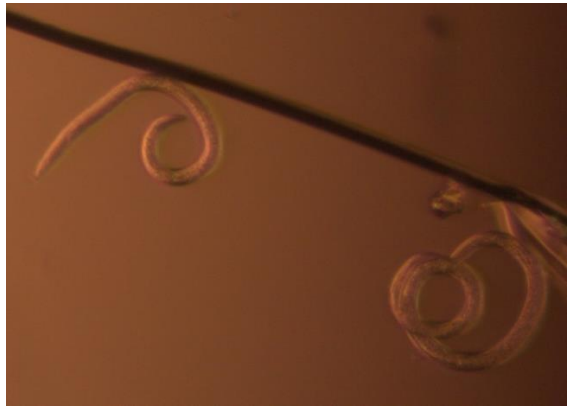
***Dorylamid* sp**



***Pratylenchus* sp**



***Trophotylenchulus* sp**



***Helicotylenchus* sp**

Helicotylenchus population in the rhizosphere soils of healthy plants was observed only during July-August. During other two seasons there was no population of this nematode in the rhizosphere soils of healthy plants. During July-August, the three different categories of plant were on par statistically for the population of *Helicotylenchus* species in the rhizosphere soil. During February-March there was no significant difference between the rhizosphere soils of apparently healthy and yellowing affected plants. During October-November, apparently healthy plants recorded highest population.

4.5.2.5 Dorylamid sp.

Dorylamid sp. were observed in the rhizosphere soils of yellowing affected and apparently healthy plants which were statistically on par and there was no population of *Dorylamid* sp. in the rhizosphere soils of healthy plants during the course of study (table 56). The population of *Dorylamid* was highest during October-November which was statistically on par with February-March. During July-August population of *Dorylamid*s was significantly lowest in the rhizosphere soils.

4.5.2.6 Trophotylenchulus sp.

The population of *Trophotylenchulus* sp was also observed in the rhizosphere soils of the three categories of the plant (table 57). The population was significantly lowest in healthy plants and highest in yellowing affected plants. Seasonal variation showed that population of *Trophotylenchulus* was significantly lowest during July-August and highest during February-March.

4.5.3 Nematode population in 5 g root (number/5 g root)

Nematode population was significantly highest (119.93) in the roots of yellowing affected plants and significantly lowest (2.77) in the roots of healthy plants (table 58).

Seasonal variation showed that the nematode population was significantly low (27.93) during July-August and the other two seasons were statistically on par. During all the three seasons nematode population was lowest in the roots of healthy plants followed by apparently healthy and yellowing affected plants. During July-August, nematode population was lowest in the roots of apparently healthy and yellowing affected plants compared to October-November and February-March.

However, during October-November and February-March nematode count was significantly highest in the roots of yellowing affected plants compared to apparently healthy and healthy plants.

4.5.3.1 Number of galls in 5g roots

As can be seen from table 59 healthy plants showed significantly lower number of galls on roots compared to apparently healthy and yellowing affected plants. The number of galls in the roots was significantly highest in yellowing affected plants.

During July-August and October-November both apparently healthy and yellowing affected plants were statistically on par for number of galls on roots. In healthy plants significantly lowest number galls were recorded. During, February-March healthy plants showed significantly lowest number of galls whereas apparently healthy and yellowing affected plants were statistically on par.

4.5.3.2 Population Meloidogyne incognita in 5g roots

As can be seen from table 60 *M. incognita* population was absent in healthy plants. The population of nematodes in 5 g root samples were statistically on par in apparently healthy and yellowing affected plants during all the three seasons under study.

Considering different categories of nematodes, *Meloidogyne incognita* was predominantly observed in the roots of yellowing affected plants.

4.5.3.3 Radopholus similis population in 5g roots

Significantly highest number of *Radopholus similis* population was recorded in roots of yellowing affected plants when compared to apparently healthy and healthy plants (table 61).

As in the case of population of *M. incognita*, *Radopholus similis* population also was zero in the roots of healthy black pepper plants (Table 61). Yellowing affected and apparently healthy plants were on par with respect to *Radopholus similis* population during July-August. However, during October-November and February-March yellowing affected plants showed significantly highest population of *Radopholus similis*.

4.5.4 Seasonal variation in the nematode population in roots

As in the case of nematode population in the soil total nematode population in roots also was significantly highest during October-November and population was significantly lowest during July-August compared to October-November and February-March (table 58).

Number of galls in the roots was also significantly highest during October-November and lowest during July-August (table 59).

Population of *M. incognita* in the roots was significantly highest during October-November and lowest during July-August (table 60).

Population of burrowing nematode *Radopholus similis* in the roots was also lowest during July-August and population was statistically on par during October-November and February-March (table 61).

Table 55. *Helicotylenchus sp.* population in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	1.40 (1.29)	0.60 (0.94)	**1.00 (1.11)**	28.40 (5.35)	9.40 (3.12)	**18.90 (4.23)**	11.20 (3.36)	8.80 (2.93)	**10.00 (3.14)**
A. H	1.80 (1.47)	1.20 (1.21)	**1.50 (1.34)**	60.40 (7.76)	3.40 (1.91)	**31.90 (4.84)**	13.20 (3.55)	1.80 (1.37)	**7.50 (2.46)**
Healthy	0.00 (0.71)	0.20 (0.81)	**0.10 (0.76)**	0.00 (0.71)	0.00 (0.71)	**0.000 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Year mean	**1.06 (1.15)**	**0.66 (0.99)**		**29.60 (4.60)**	**4.267 (1.91)**		**8.133 (2.54)**	**3.533 (1.67)**	
Seasonal mean	**0.86 (1.07)**			**16.93 (3.26)**			**5.83 (2.10)**		

Category (C) / Year	**2017 (B1)**	**2018 (B2)**	**Treatment Mean**
Yellowing affected plants	13.66 (3.33)	6.26 (2.33)	**9.96 (2.83)**
Apparently healthy plants	25.13 (4.26)	2.13 (1.50)	**13.63 (2.88)**
Healthy plants	0.00 (0.71)	0.06 (0.74)	**0.03 (0.72)**
Category /Year mean	**12.93 (2.76)**	**2.82 (1.52)**	

	CD (0.05)		**CD (0.05)**
Season- (A)	**0.29**	Season x Year (A x B)	**0.42**
Year – (B)	**0.24**	Season x Category (A x C)	**0.51**
Category – (C)	**0.29**	Year x Category (B x C)	**0.42**
		Season x Year x Category (A x B x C)	**0.72**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 56. *Dorylamid sp.* population in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	0.80 (1.04)	0.00 (0.71)	**0.40 (0.87)**	6.60 (2.66)	2.00 1.47)	**4.30 (2.06)**	4.60 (2.17)	2.00 (1.50)	**3.30 (1.83)**
A. H	0.00 (0.71)	0.60 (1.01)	**0.30 (0.86)**	5.60 (2.42)	1.40 (1.27)	**3.50 (1.85)**	2.60 (1.72)	2.20 (1.61)	**2.40 (1.67)**
Healthy	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Year mean	**0.26 (0.82)**	**0.20 (0.81)**		**4.06 (1.93)**	**1.13 (1.15)**		**2.40 (1.53)**	**1.40 (1.27)**	
Seasonal mean	**0.23 (0.81)**			**2.60 (1.54)**			**1.90 (1.40)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	4.00 (1.95)	1.33 (1.22)	**2.66 (1.59)**
Apparently healthy plants	2.73 (1.62)	1.40 (1.30)	**2.06 (1.46)**
Healthy plants	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Category /Year mean	**2.24 (1.43)**	**0.91 (1.08)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.18**	Season x Year (A x B)	**0.25**
Year – (B)	**0.14**	Season x Category (A x C)	**0.31**
Category – (C)	**0.18**	Year x Category (B x C)	**0.25**
		Season x Year x Category (A x B x C)	**0.44**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 57. *Trophotylenchulus* sp. in rhizosphere soils of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	1.60 (1.39)	0.20 (0.81)	**0.90 (1.10)**	5.20 (2.33)	1.00 (1.16)	**3.10 (1.74)**	8.20 (2.94)	0.80 (1.08)	**4.50 (2.01)**
A. H	0.80 (1.04)	0.60 (0.98)	**0.70 (1.01)**	3.00 (1.78)	0.40 (0.91)	**1.70 (1.34)**	6.20 (2.53)	1.20 (1.16)	**3.70 (1.84)**
Healthy	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	1.00 (1.03)	**0.50 (0.87)**
Year mean	**0.80 (1.04)**	**0.26 (0.83)**		**2.73 (1.60)**	**0.46 (0.92)**		**4.80 (2.06)**	**1.00 (1.09)**	
Seasonal mean	**0.53 (0.94)**			**1.60 (1.26)**			**2.90 (1.58)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	5.00 (2.22)	0.66 (1.02)	**2.83 (1.62)**
Apparently healthy plants	3.33 (1.78)	0.73 (1.02)	**2.03 (1.40)**
Healthy plants	0.00 (0.71)	0.33 (0.81)	**0.16 (0.76)**
Category /Year mean	**2.77 (1.57)**	**0.57 (0.95)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.21**	Season x Year (A x B)	**0.30**
Year – (B)	**0.17**	Season x Category (A x C)	**0.37**
Category – (C)	**0.21**	Year x Category (B x C)	**0.30**
		Season x Year x Category (A x B x C)	**0.53**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 58. Nematode population in roots of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	65.60	24.80	**45.20**	231.80	115.20	**173.50**	146.80	133.60	**140.20**
A. H	49.80	22.40	**36.10**	160.80	91.20	**126.00**	91.40	84.00	**87.70**
Healthy	1.60	3.40	**2.50**	2.80	3.40	**3.10**	2.40	3.00	**2.70**
Year mean	**39.00**	**16.86**		**131.80**	**69.93**		**80.20**	**73.53**	
Seasonal mean	**27.93**			**100.86**			**76.86**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	148.06	91.20	**119.63**
Apparently healthy plants	100.66	65.86	**83.26**
Healthy plants	2.26	3.26	**2.76**
Category /Year mean	**83.66**	**53.44**	

	CD (0.05)		CD (0.05)
Season- (A)	**15.52**	Season x Year (A x B)	**21.95**
Year – (B)	**12.67**	Season x Category (A x C)	**26.88**
Category – (C)	**15.52**	Year x Category (B x C)	**21.95**
		Season x Year x Category (A x B x C)	**38.02**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Table 59. Number of galls in roots of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	14.80 (3.83)	14.20 (3.76)	**14.50 (3.80)**	61.60 (7.86)	55.00 (7.40)	**58.30 (7.63)**	37.20 (6.00)	37.20 (6.08)	**37.20 (6.04)**
A. H	14.80 (3.74)	8.40 (2.96)	**11.60 (3.35)**	55.60 (7.31)	47.60 (6.87)	**51.60 (7.09)**	33.20 (5.65)	22.40 (4.72)	**27.80 (5.18)**
Healthy	0.60 (0.98)	0.00 (0.71)	**0.30 (0.84)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	3.20 (1.88)	0.00 (0.71)	**1.60 (1.29)**
Year mean	**10.06 (2.85)**	**7.53 (2.47)**		**39.06 (5.29)**	**34.20 (4.99)**		**24.53 (4.51)**	**19.86 (3.83)**	
Seasonal mean	**8.80 (2.66)**			**36.63 (5.14)**			**22.20 (4.17)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	37.86 (5.90)	35.46 (5.75)	**36.66 (5.82)**
Apparently healthy plants	34.53 (5.57)	26.13 (4.85)	**30.33 (5.21)**
Healthy plants	1.26 (1.19)	0.00 (0.71)	**0.63 (0.95)**
Category /Year mean	**24.55 (4.22)**	**20.53 (3.77)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.47**	Season x Year (A x B)	**0.67**
Year – (B)	**0.38**	Season x Category (A x C)	**0.82**
Category – (C)	**0.47**	Year x Category (B x C)	**0.67**
		Season x Year x Category (A x B x C)	**1.16**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Table 60. Number of *Meloidogyne incognita* in roots of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	36.40 (5.90)	35.20 (5.87)	**35.80 (5.89)**	101.60 (9.69)	88.00 (9.27)	**94.80 (9.48)**	77.20 (8.60)	79.40 (8.87)	**78.30 (8.74)**
A. H	21.80 (4.61)	21.20 (4.15)	**21.50 (4.38)**	101.60 (9.80)	69.60 (8.17)	**85.60 (8.99)**	74.80 (8.67)	44.00 (6.53)	**59.40 (7.60)**
Healthy	0.00 (0.71)	0.00 (0.71)	**0.00 (4.38)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Year mean	**19.40 (3.74)**	**18.80 (3.58)**		**67.73 (6.73)**	**52.53 (6.05)**		**50.66 (5.99)**	**41.13 (5.37)**	
Seasonal mean	**19.10 (3.66)**			**60.13 (6.39)**			**45.90 (5.68)**		

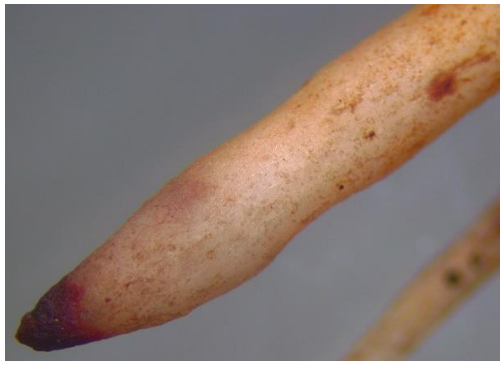
Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	71.73 (8.06)	67.53 (8.00)	**69.63 (8.03)**
Apparently healthy plants	66.06 (7.69)	44.93 (6.29)	**55.50 (6.99)**
Healthy plants	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Category /Year mean	**45.93 (5.49)**	**37.48 (5.00)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.78**	Season x Year (A x B)	**1.11**
Year – (B)	**0.64**	Season x Category (A x C)	**1.36**
Category – (C)	**0.78**	Year x Category (B x C)	**1.11**
		Season x Year x Category (A x B x C)	**1.93**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Plate 11. Nematode infection in roots of yellowing affected black pepper plants



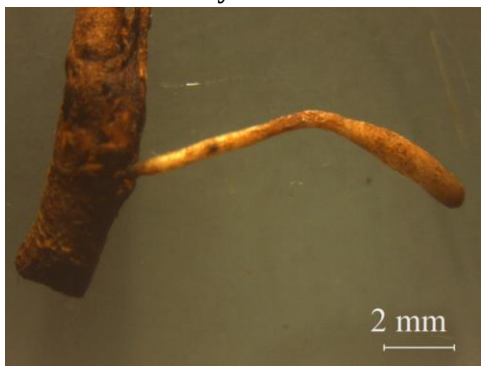
Healthy root tip

Tumors on primary roots

Galls on tertiary roots

Necrosis at root tip

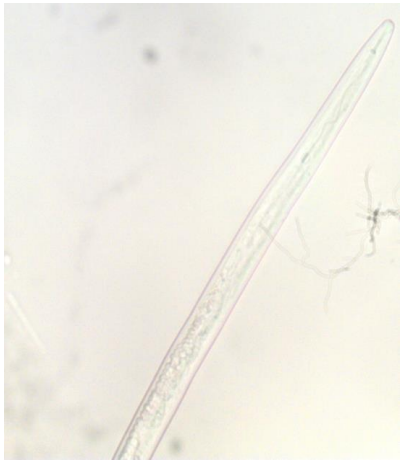
Incidence of galls on feeder roots



Gall at end of root tips

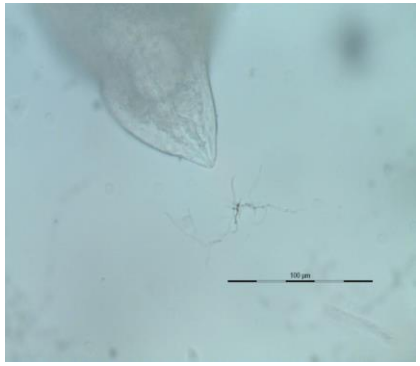
Plate 12. Identification of *M. incognita* in yellowing affected black pepper plants

M.incognita

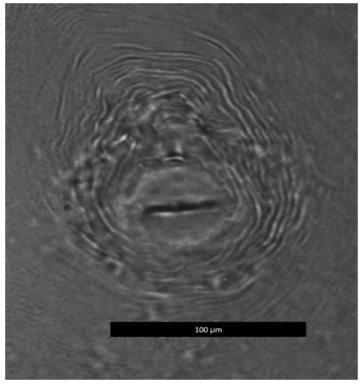


Head region

Tail region



Adult female snout portion



Perennial pattern

4.5.5 Per cent incidence of nematodes in roots

As can be seen from table 62 during October- November *M. incognita* population was significantly high in apparently healthy plants compared to yellowing affected plants. During February-March *R. similis* population was significantly high in yellowing affected plants. However, both *M. incognita* and *Radopholus similis* population was observed during all the three seasons.

4.6 EXPERIMENT VI

4.6.1 Identification of root mealy bugs

Roots and soil samples were analysed during three seasons for two years in healthy, apparently healthy and yellowing affected plants. Examination of roots of experimental plants and rhizosphere soil did not show the presence of root mealy bugs or any other insect, which can cause damage to root or yellowing in the plant. The possibility of any insect including root mealy bug involved in causing yellowing of black pepper in the experimental area was ruled out.

4.7 EXPERIMENT VII

4.7.1 Correlation with weather variables

4.7.1.1 Correlation of yellowing with weather variables

There was significant positive correlation of yellowing with maximum temperature (table 63). However minimum temperature showed significant negative correlation with yellowing. Relative humidity and rainfall also were significantly and negatively correlated with yellowing.

Table 61. Number of *Radopholus similis sp* in roots of yellowing affected, apparently healthy and healthy black pepper plants

Category / Year	July – August A1			October – November A2			February – March A3		
	2017 B1	2018 B2	Category mean	2017 B1	2018 B2	Category mean	2018 B1	2019 B2	Category mean
Y	6.40 (2.53)	9.80 (3.19)	**8.10 (2.86)**	75.40 (8.50)	59.80 (7.68)	**67.60 (8.09)**	73.00 (8.20)	45.40 (6.76)	**59.20 (7.48)**
A. H	6.00 (2.43)	9.40 (3.05)	**7.70 (2.74)**	42.00 (6.11)	35.00 (5.59)	**38.50 (5.85)**	19.60 (4.23)	35.80 (5.98)	**27.70 (5.11)**
Healthy	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Year mean	**4.13 (1.89)**	**6.40 (2.31)**		**39.13 (5.10)**	**31.60 (4.66)**		**30.86 (4.38)**	**27.06 (4.48)**	
Seasonal mean (A)	**5.26 (2.10)**			**35.36 (4.886)**			**28.96 (4.43)**		

Category (C) / Year	2017 (B1)	2018 (B2)	Treatment Mean
Yellowing affected plants	51.60 (6.41)	38.33 (5.88)	**44.96 (6.14)**
Apparently healthy plants	22.53 (4.25)	26.73 (4.87)	**24.63 (4.56)**
Healthy plants	0.00 (0.71)	0.00 (0.71)	**0.00 (0.71)**
Category /Year mean	**24.71 (3.79)**	**21.68 (3.82)**	

	CD (0.05)		CD (0.05)
Season- (A)	**0.68**	Season x Year (A x B)	**0.96**
Year – (B)	**0.55**	Season x Category (A x C)	**1.18**
Category – (C)	**0.68**	Year x Category (B x C)	**0.96**
		Season x Year x Category (A x B x C)	**1.66**

Yellowing affected plants (Y), Apparently healthy plants (A.H), Healthy plants (H)

Fig. in the parenthesis are SQRT transformed values calculated using the formula $\sqrt{(x+0.5)}$

Plate 13. *Radopholus similis* damage to roots in yellowing affected black pepper plants

Root lesion

Severely damaged mature roots

Bark decay

Swelling at nodes

Table 62. Per cent incidence of nematodes in roots of yellowing affected, apparently healthy and healthy black pepper plants

Seasons	Years mean	Nematode population in root	*Meloidogyne incognita*	*Radopholus similis*
July – August	Yellowing affected plants	45.20	35.80 (79.20)	8.10 (17.92)
	Apparently healthy plants	36.10	21.50 (59.56)	7.70 (21.33)
	Healthy plants	2.50	0.00 (0.00)	0.00 (0.00)
October – November	Yellowing affected plants	173.50	94.80 (54.64)	67.60 (38.96)
	Apparently healthy plants	126.00	85.60 (67.94)	38.50 (30.56)
	Healthy plants	3.10	0.00 (0.00)	0.00 (0.00)
February – March	Yellowing affected plants	140.20	78.30 (55.85)	67.60 (48.22)
	Apparently healthy plants	87.70	59.40 (67.73)	38.50 (43.90)
	Healthy plants	2.70	0.00 (0.00)	0.00 (0.00)
CD (0.05)		**26.88**	**23.00**	**15.58**

4.7.1.2 Correlation of yellowing and soil nutrients

The correlation of different rhizosphere soil characters with yellowing is given in table 64. It can be seen from the table that the soil pH, EC and Organic Carbon were significantly and positively correlated with yellowing. There was no significant correlation of N, P, Mg, S, Fe, Mn, Zn, Cu and B content of rhizosphere soil with yellowing in black pepper. K, Ca and Al content of rhizosphere soils showed significant negative correlation yellowing.

4.7.1.3 Correlation of yellowing and tissue nutrients

Tissue analysis of yellowing affected, apparently healthy and healthy plants showed negative correlation of yellowing with N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Al content in plant samples. (table 65).

4.7.1.4 Correlation of soil nematode population with weather variables

The soil nematode population showed negative correlation with minimum temperature, relative humidity and rainfall. Yellowing was positively correlated with the soil nematode population.

Table 63. Correlation of yellowing with weather variables during period of study.

	Yellowing	Maximum temperature	Minimum temperature	Relative humidity	Rainfall
Yellowing	1.000				
Maximum temperature	0.144*	1.000			
Minimum temperature	-0.132*	0.097NS	1.000		
Relative humidity	-0.151*	-0.996**	-0.059NS	1.000	
Rainfall	-0.178**	-0.897**	0.144*	0.912**	1.000

Table 64. Correlation of yellowing with rhizosphere soils nutrients of healthy, apparently healthy and yellowing affected plants during study

	Yellowing	pH	EC	O.C	Fe	Mn	Z	Cu	B	N	P	K	Ca	Mg	S	Al
Yellowing	1.000															
pH	0.183**	1.000														
EC	0.150*	0.147*	1.000													
O.C	0.288**	0.182**	0.038NS	1.000												
Fe	-0.058NS	-0.117NS	-0.039NS	0.031NS	1.000											
Mn	0.077NS	-0.163**	-0.157**	0.030NS	0.012NS	1.000										
Zu	-0.064NS	0.227**	-0.167**	0.166**	0.109NS	-0.102NS	1.000									
Cu	0.025NS	0.088NS	-0.021NS	0.093NS	-0.131*	-0.054NS	0.225**	1.000								
B	0.082NS	0.108NS	-0.104NS	0.030NS	0.004NS	0.046NS	0.125*	-0.039NS	1.000							
N	-0.111NS	-0.051NS	-0.250**	-0.095NS	0.145*	0.136*	0.000NS	-0.053NS	0.026NS	1.000						
P	-0.067NS	-0.088NS	-0.015NS	-0.123*	0.059NS	0.047NS	-0.051NS	-0.033NS	0.017NS	0.012NS	1.000					
K	-0.159**	0.122*	-0.104NS	-0.123*	-0.049NS	-0.148*	0.412**	0.229**	0.154*	0.109NS	0.037NS	1.000				
Ca	-0.270**	0.102NS	-0.284**	-0.099NS	0.048NS	-0.012NS	0.443**	0.230**	0.127*	0.100NS	-0.082NS	0.450**	1.000			
Mg	0.050NS	0.263**	-0.087NS	0.101NS	-0.042NS	-0.004NS	0.199**	0.104NS	0.137*	0.120*	-0.097NS	0.246**	0.208**	1.000		
S	0.032NS	0.082NS	-0.293**	-0.048NS	0.070NS	0.041NS	0.185**	0.140*	0.230**	0.060NS	0.056NS	0.129*	0.240**	0.125*	1.000	
Al	-0.233**	0.045NS	-0.044NS	-0.293**	-0.010NS	0.112NS	-0.054NS	-0.059NS	0.035NS	0.129*	0.161**	0.016NS	0.112NS	-0.058NS	-0.039NS	1.000

Table 65. Correlation of yellowing with tissue analysis of yellowing affected, apparently healthy and healthy plants during study

	Yellowing	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Al
Yellowing	1.000												
N	-0.128*	1.000											
P	-0.276**	0.209**	1.000										
K	-0.343**	0.268**	0.328**	1.000									
Ca	-0.311**	0.507**	0.200**	0.223**	1.000								
Mg	-0.162**	0.048^{NS}	0.095^{NS}	0.074^{NS}	-0.007^{NS}	1.000							
S	-0.348**	-0.081^{NS}	0.196**	0.172**	-0.007^{NS}	0.203**	1.000						
Fe	-0.311**	-0.010^{NS}	0.279**	0.300**	-0.033^{NS}	0.126*	0.276**	1.000					
Mn	-0.346**	0.374**	0.296**	0.148*	0.524**	0.027^{NS}	0.080^{NS}	0.077^{NS}	1.000				
Zn	-0.217**	0.316**	0.175**	0.189**	0.343**	-0.037^{NS}	0.282**	-0.097^{NS}	0.384**	1.000			
Cu	-0.179**	0.474**	0.223**	0.352**	0.421**	0.004^{NS}	0.057^{NS}	0.190**	0.408**	0.283**	1.000		
B	-0.263**	0.367**	0.196**	0.117^{NS}	0.439**	-0.026^{NS}	0.046^{NS}	-0.008^{NS}	0.469**	0.279**	0.410**	1.000	
Al	-0.364**	-0.016^{NS}	0.226**	0.205**	0.048^{NS}	0.089^{NS}	0.120*	0.410**	0.131*	-0.066^{NS}	0.012^{NS}	-0.013^{NS}	1.000

Table 66. Correlation of soil nematode population with weather variables

	Yellowing	Soil nematodes population	Maximum temperature	Minimum Temperature	Relative humidity	Rainfall
Yellowing	1.000					
Soil nematodes population	0.435**	1.000				
Maximum temperature	0.162NS	0.188NS	1.000			
Minimum temperature	-0.089NS	-0.294**	0.097NS	1.000		
Relative humidity	-0.158NS	-0.213*	-0.996**	-0.059NS	1.000	
Rainfall	-0.180NS	-0.307**	-0.897**	0.144NS	0.912**	1.000

Discussion

5. DISCUSSION

The present study entitled, "Investigations on yellowing of black pepper (*Piper nigrum* L.)" comprising of laboratory and field experiments was carried out during the period 2016-2019 at Department of Plantation crops and Spices, Radio Tracer Laboratory and Department of Plant Pathology, College of Horticulture and Banana Research Station, Kannara. Part of laboratory analysis was also conducted at nematology labs of IIHR, Bangalore and ICAR-Central Potato Research Station Ooty. The study was undertaken to find the role of soil nutrients, plant pathogens, insects and nematodes if any in causing yellowing of black pepper.

A comprehensive purposive sampling survey was conducted in the pepper growing tracts of Thrissur so as to initially identify the intensity and spread of yellowing. Based on the survey, six different locations were selected for conducting the experiment.

In the selected tracts, samples were specifically identified and collected in a scale that included a. healthy, b. apparently healthy and c. yellowing affected plants. Fifteen yellowing affected plants and fifteen apparently healthy plants were selected from the same field. Healthy plants were selected from fields without yellowing symptoms. Black pepper varieties like Panniyur 1, Panniyur 2, Panniyur 3, Karimunda, and Vijay were included in the study.

5.1 EXPERIMENT I

5.1.1 Study on the initiation and development of yellowing

5.1.1.1 Symptomatological studies

Symptoms of yellowing generally developed at the fag end of monsoon. Yellowing was observed either on the leaves at the top of pepper column or (and) the bottom of the column. Sometimes yellowing appeared in a group of leaves together. Both young and mature leaves closely together were found yellow. According to

George *et al*. (2005), the lower leaves of black pepper turned yellowish but the upper canopy of affected plants remained relatively green in nitrogen deficient plants. In severe cases, leaves of the entire plant showed a characteristic yellow to orange yellow discoloration.

In some leaves the base of lamina near petiole was yellow which faded to tip of lamina and margins. In some other cases, interveinal yellowing was observed. Sometimes yellowing was observed in one or two leaves on a branch where as in some other cases all the leaves on lateral branches were yellow.

Another type of yellowing was observed at the time of fruit maturity during October-November. In this type of yellowing recovery was observed with the onset of monsoon. This clearly showed that rain, high humidity and soil moisture had a suppressive role on yellowing. Symptoms of yellowing started as mild discoloration of leaves which was spreading to whole plant. Yellowing was seen on leaves of all stages.

The varying symptoms suggested that there were multiple factors involved in the development and spread of yellowing. The analyses of nutrients in the leaf tissue of yellowing affected plants indicated deficiency of some of the nutrients in the leaf tissue. Deficiency of different elements at varying levels, coupled with infection from nematodes could be the reason for varying pattern of yellowing in the plants.

In case of mild yellowing there was recovery during July-August with the onset of monsoon. There was reduction in root nodules and fresh roots developed during monsoon. Yellowing gradually increased and severely affected plants failed to recover. There was no flushing or flowering in plants. Such plants died in 2-3 years.

5.1. 2 Yield and yield contributing characters

Analysis of yield and yield contributing characters in healthy, apparently healthy and yellowing affected black pepper plants showed that for all the characters

like height of bearing column, spread N-S and, E-W and number of laterals per unit area healthy plants showed a higher positive value, compared to yellowing affected plants, even though it was not statistically significant (table 7, 8, 9 and 10, fig 1, 2, 3 and 4). In the case of number of spikes per unit area, varieties Panniyur 3, Karimunda and Vijay were affected significantly (table 11 and fig 5). Other two varieties studied viz., Panniyur 1, and Panniyur 2 also showed lower number of spikes per unit area even though statistically not significant. In the case of yield per plant, there was significant reduction in yield in yellowing affected plants compared to healthy in all varieties except Vijay (table 12 and fig 6). In the variety Vijay also yield reduction was observed in yellowing affected plants even though not significant. As the data on yield and yield contributing characters suggest there was an indication of reduction in growth, yield contributing characters and yield even though not significant in some cases. Yellowing was reported as a slowly debilitating disease in black pepper. That could be the reason for a slow reduction in growth and yield characters. Most of the yellowing noticed in the experimental plants were in the early/ initial stages of yellowing, which recovered in the subsequent rainy seasons. Similar reduction in the yield in yellowing affected black pepper plants were reported by Sreekumar (2015) in Wayanad district of Kerala. Symptoms of yellowing were seen which spread to adjacent plants and in severe conditions the whole plant turned lemon yellow in colour.

An analysis of the yield and yield contributing characters showed that in the experimental plants studied, there was no definite pattern on the effect of yellowing on characters like height of bearing column, plant spread in N-S and E-W direction and number of laterals per unit area.

Plate. 14. Symptoms of yellowing on leaves

Healthy leaf

Yellowing one half of leaf

Severe interveinal yellowing from leaf base

Interveinal yellowing from leaf tip

Yellowing and browning from leaf tip

Interveinal yellowing from middle leaf and margins

Plate. 15. Symptoms of yellowing on leaves

Overall pale yellow discoloration

Intense yellowing of a few leaves

Yellowing at leaf base

Dull yellow discoloration from leaf base

Over all yellowing

Lemon yellow discoloration of whole leaf

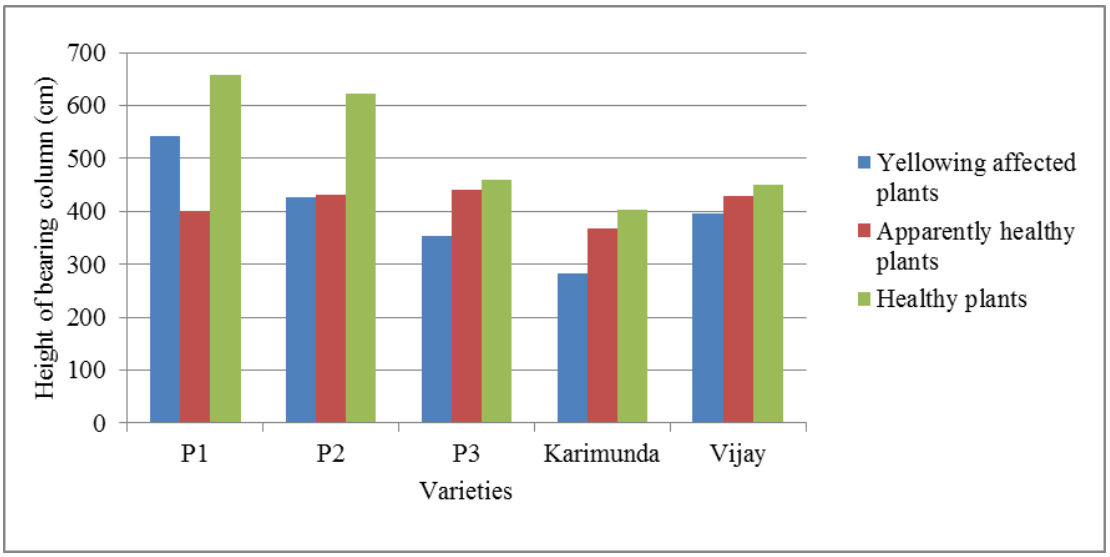


Figure 1. Height of bearing column (cm) in healthy, apparently healthy and yellowing affected black pepper varieties

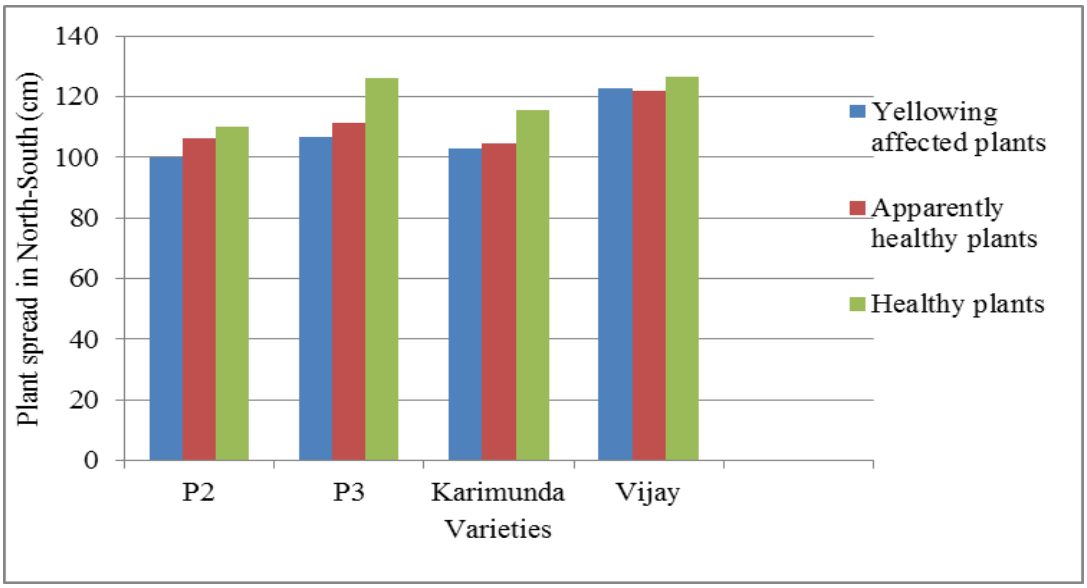


Figure 2. Plant spread in North-South direction (cm) in healthy, apparently healthy and yellowing affected black pepper varieties

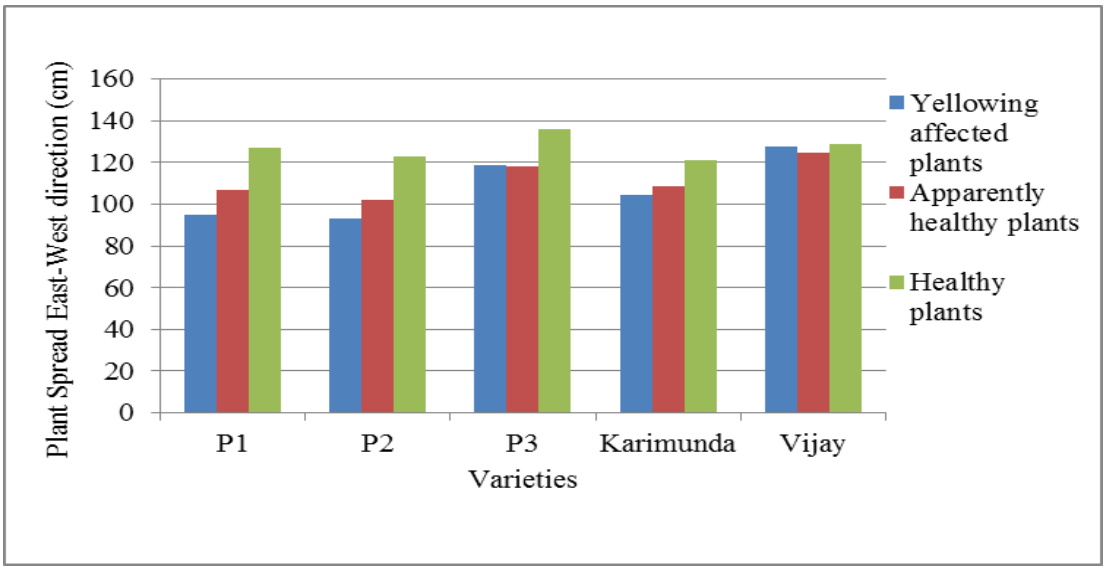


Figure 3. Plant spread in East-West direction (cm) in healthy, apparently healthy and yellowing affected black pepper varieties

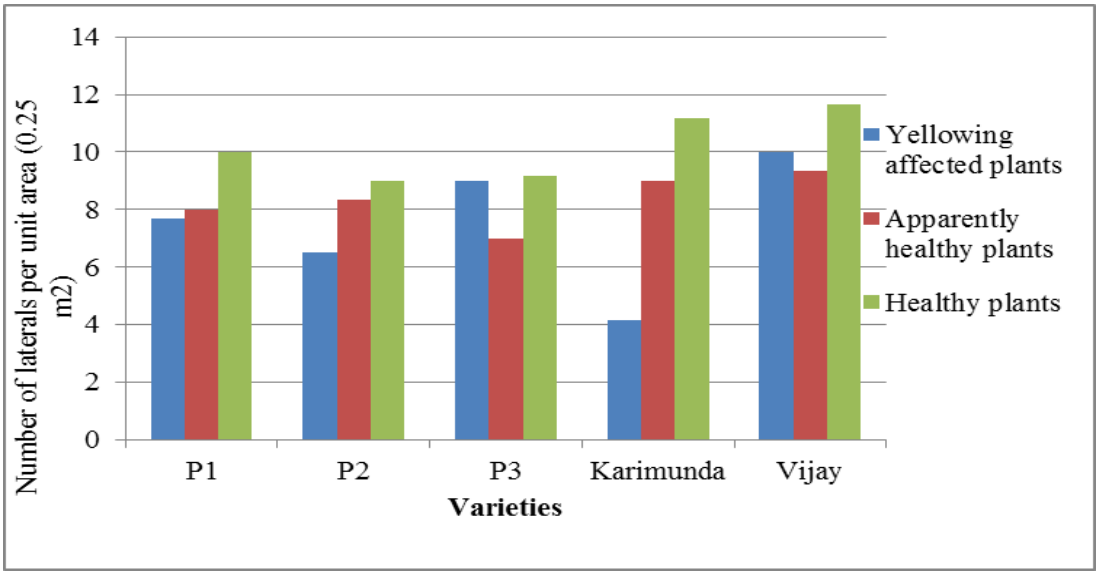


Figure 4. Number of laterals per unit area in healthy, apparently healthy and yellowing affected black pepper varieties

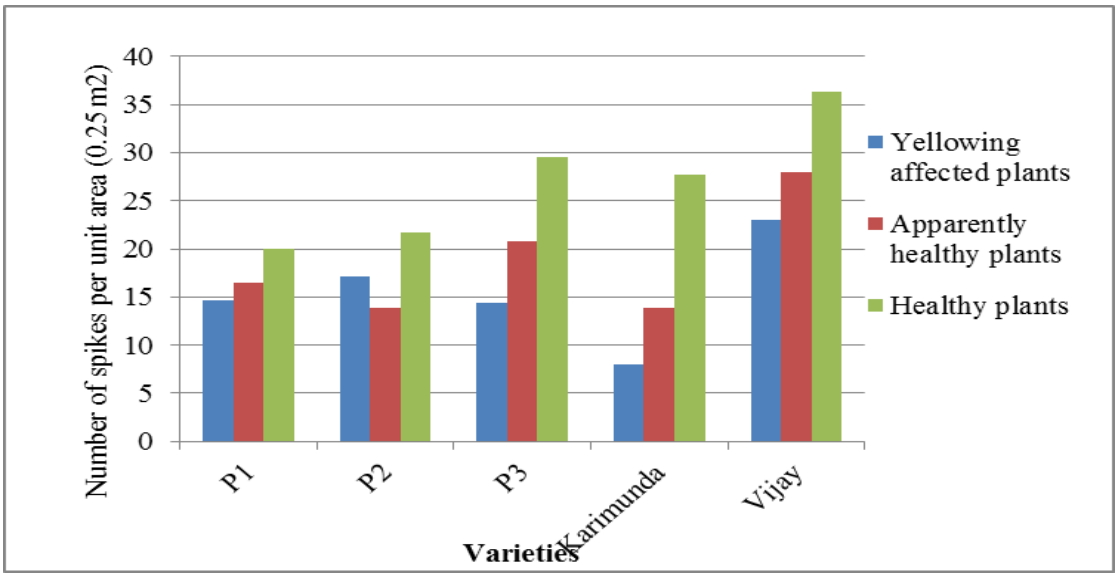


Figure 5. Number of spikes per unit area in healthy, apparently healthy and yellowing affected black pepper varieties

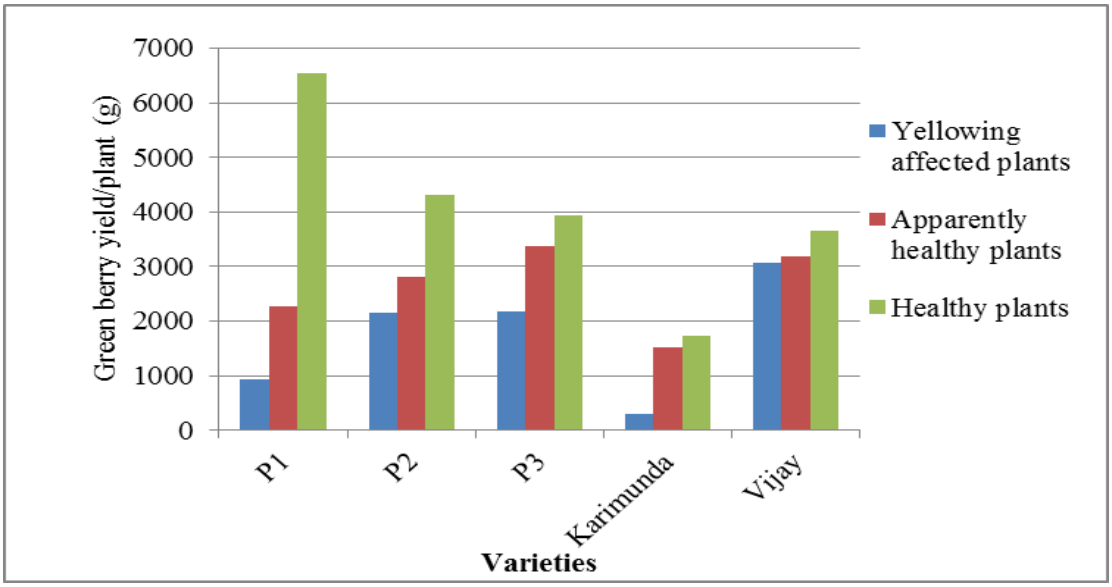


Figure 6. Green berry yield per plant (g) in healthy, apparently healthy and yellowing affected of black pepper varieties

5.2 EXPERIMENT-2

5.2.1 Analysis of rhizosphere soil character and their association with yellowing

5.2.1.1 pH

In the present study it was observed that rhizosphere soil of healthy plants showed significantly lower pH, compared to yellowing affected plants (table 19 and fig.7). Sreekumar (2015) and Aloka, (2016), had reported very low pH, and associated soil nutrient imbalance to be one of the main causes for yellowing in black pepper in Wayanad. However, in the present study pH of the yellowing affected gardens was in the range of 5.28 to 6.58 which is ideal pH range reported for black pepper. According to DRIS norms suggested by Hamza *et al.* (2007) optimum pH range for black pepper soils is 4.75-6.15 which supported the view that soil pH of the experimental plots were in the range suited for black pepper. In the present study yellowing of pepper cannot be attributed to very low pH of rhizosphere soil and associated soil nutrient imbalance which was a major cause of yellowing reported in Wayanad by Sreekumar, (2015) and Aloka, (2016).

5.2.1.2 Organic carbon

In the present study organic carbon content in the rhizosphere soil of experimental plants ranged from 0.77 to 1.84. According to DRIS norms suggested Hamza *et al.* (2007) optimum organic carbon content in the rhizosphere soil of black pepper was in the range of 2.00 to 7.50. In the present study it can be seen that organic carbon content of the experimental plots were lower than critical values (table 21 and fig 8). Hamza *et al*. (2007) reported that in 88 per cent of the black pepper gardens of Kerala and Karnataka, organic carbon status was below critical values. Sreekumar (2015) in a study conducted in Wayanad reported that organic carbon content was low in 21 per cent samples, medium in 38 per cent and high in 41 per cent samples. Despite this situation of poor organic carbon content in the

experimental fields in the present study, we can see that organic carbon content of the rhizosphere soil of healthy plants was significantly low in comparison to yellowing affected plants. The experimental plots were selected in farmers fields in different parts of Thrissur. The low organic matter status of healthy plot could have been the reason for low organic matter content in the rhizosphere of healthy plants. However, the higher organic matter content of rhizosphere soil was not reflected in plant health, probably due to root damage and poor absorption.

5.2.1.3 Nitrogen

As can be seen from table 22, over all nitrogen content during three seasons was significantly higher in the rhizosphere soils of healthy plants. Whereas, apparently healthy and yellowing affected plants were statistically on par. The lowest nitrogen content was noticed during July-August (279.67 kg ha^{-1}) which was statistically on par with February-March (292.45 kg ha^{-1}). The results indicated that nitrogen content in the rhizosphere soil does not have a direct role in causing yellowing in the experimental plots of the present study.

5.2.1.4 Phosphorus

Mean phosphorus content in the rhizosphere soil of experimental plots ranged from 96.11 to 153.87 ppm (table 23 and fig 9). According to DRIS norms suggested by Hamza *et al*. (2007) optimum P level for black pepper soil is 12- 96.0. Levels above 96 ppm were reported to be high. Nair, (2011) reported that heavy input of phosphatic fertilizers lead to build up of very high levels of nutrients in soils which adversely affected the absorption of micronutrients like Zn and boron by pepper.

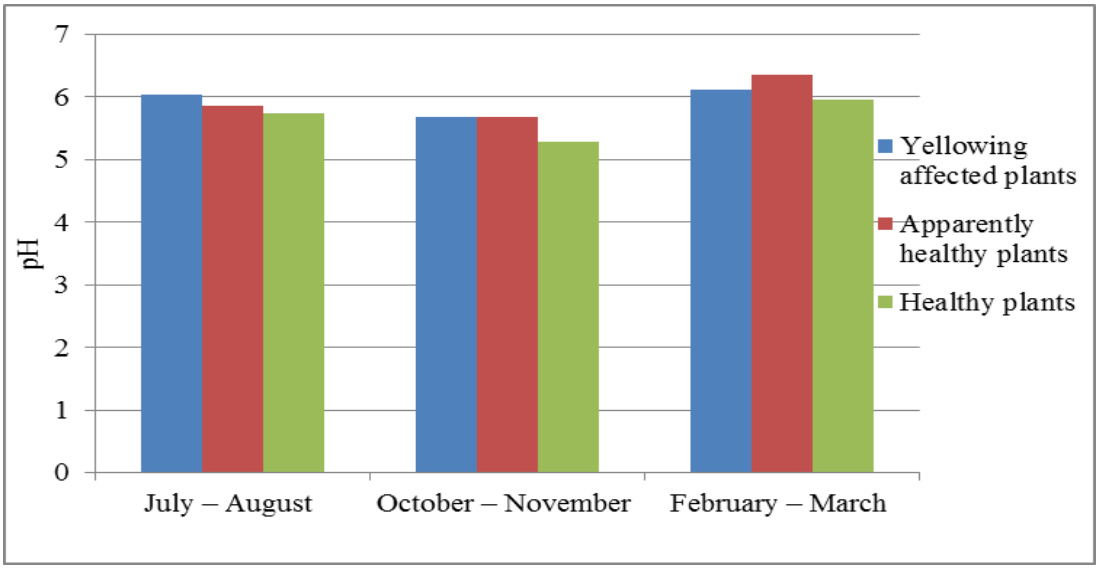


Figure 7. pH of rhizosphere soils of healthy, apparently healthy and yellowing affected plants

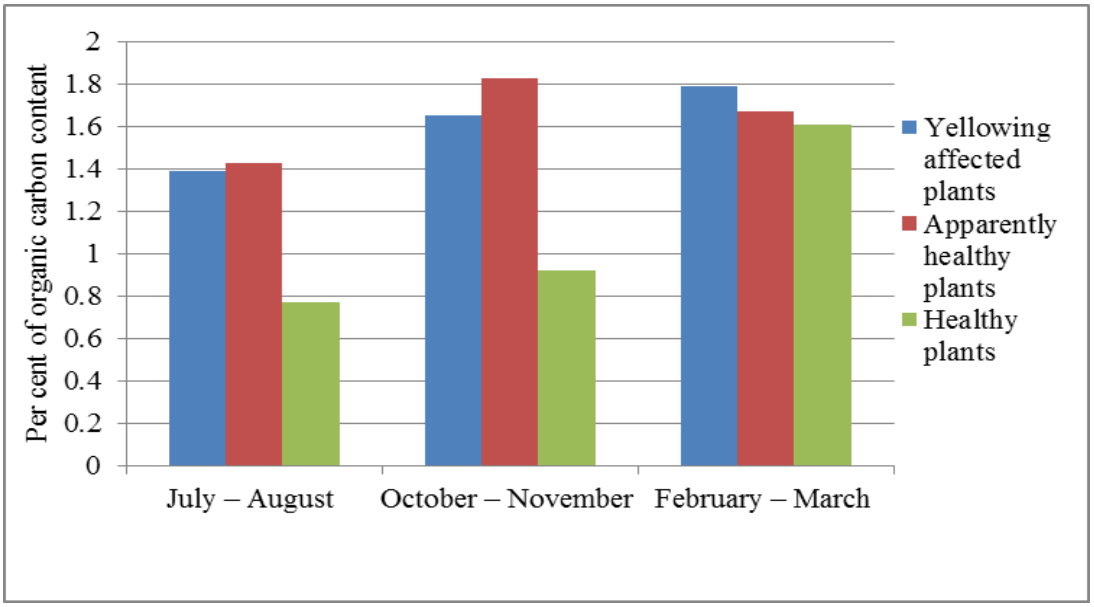


Figure 8. Organic carbon content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

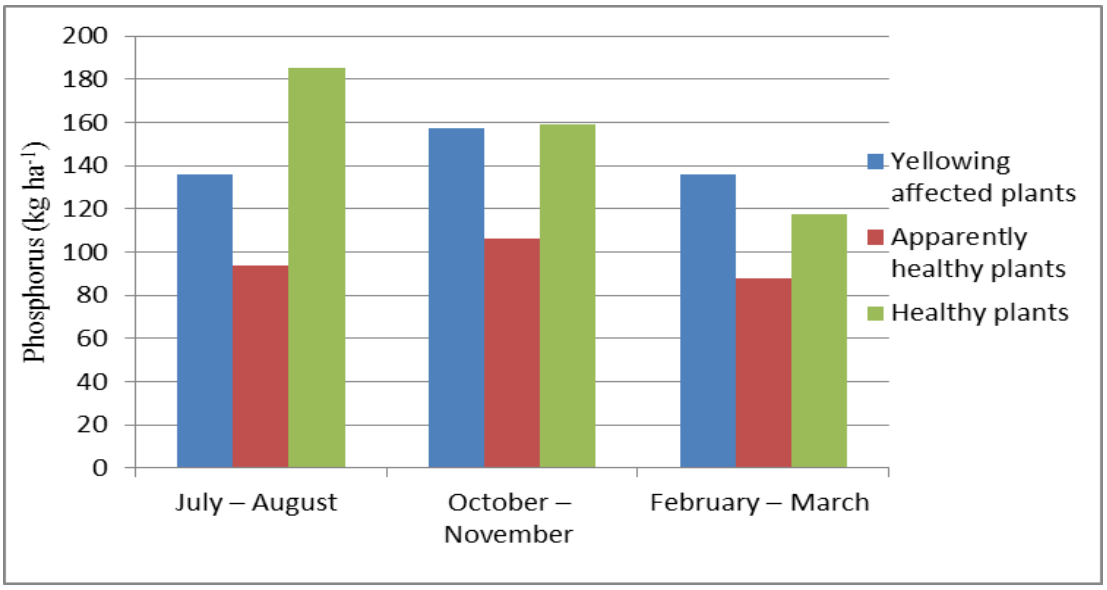


Figure 9. Phosphorous content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

5.2.1.5 Potassium

Potassium content in the rhizosphere soil was significantly higher in healthy black pepper plants (table 24 and fig 10). According to DRIS norms in black pepper, optimum level of K in soil is 91.0 to 289.0 ppm. In the present study apparently healthy and yellowing affected plants showed K content with in optimum range during July-August and September –October. During February – March, K content was high in yellowing affected and apparently healthy plants. However, the healthy plants showed potassium content in the high range throughout the period of study. However, Sreekumar (2015) reported low potassium content in the rhizosphere soil of yellowing affected plants. Sadanandan *et al*. (2000) also reported medium to high potassium content in slopes of Western Ghats of South India.

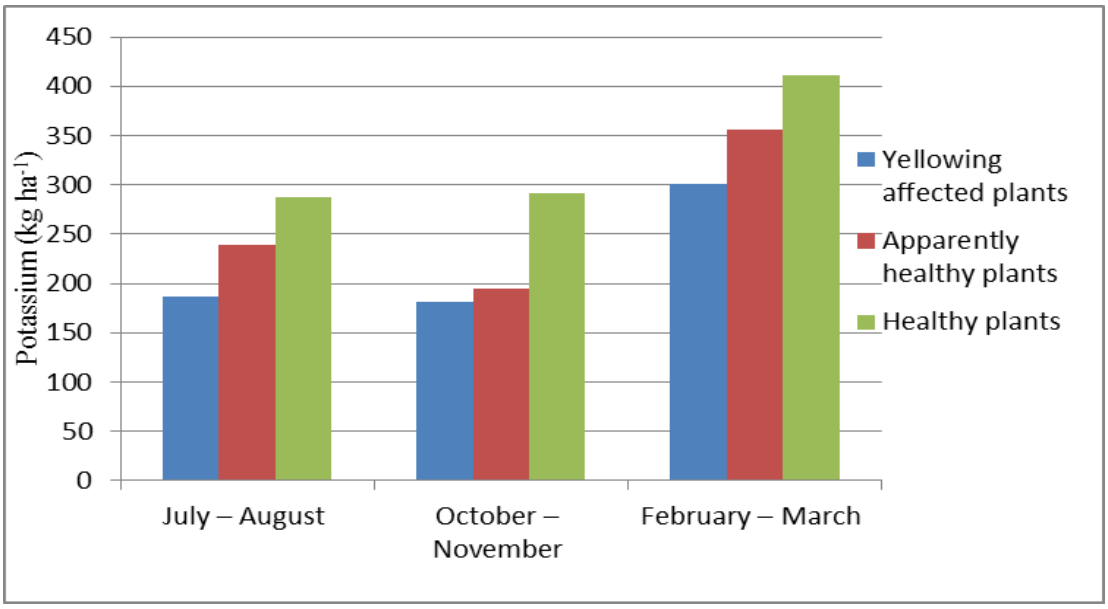


Figure 10. Potassium content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

5.2.1.6 Calcium

Calcium content in the rhizosphere soil of healthy, apparently healthy and yellowing affected plants was in the range between 768.27 to 1239.83 ppm. (table 25 fig 11) Calcium content in the experimental fields were well within the optimum range suggested by Hamza *et al*. (2007) and there was no deficiency or toxicity reported.

5.2.1.7 Magnesium

Magnesium content in the experimental fields was high in all the three categories of plants (table 26, fig 12) during February-March. During July-August and October-November magnesium content was optimum in the rhizosphere soil of yellowing affected and apparently healthy plants. This clearly indicated that there was no deficiency of magnesium in the experimental fields.

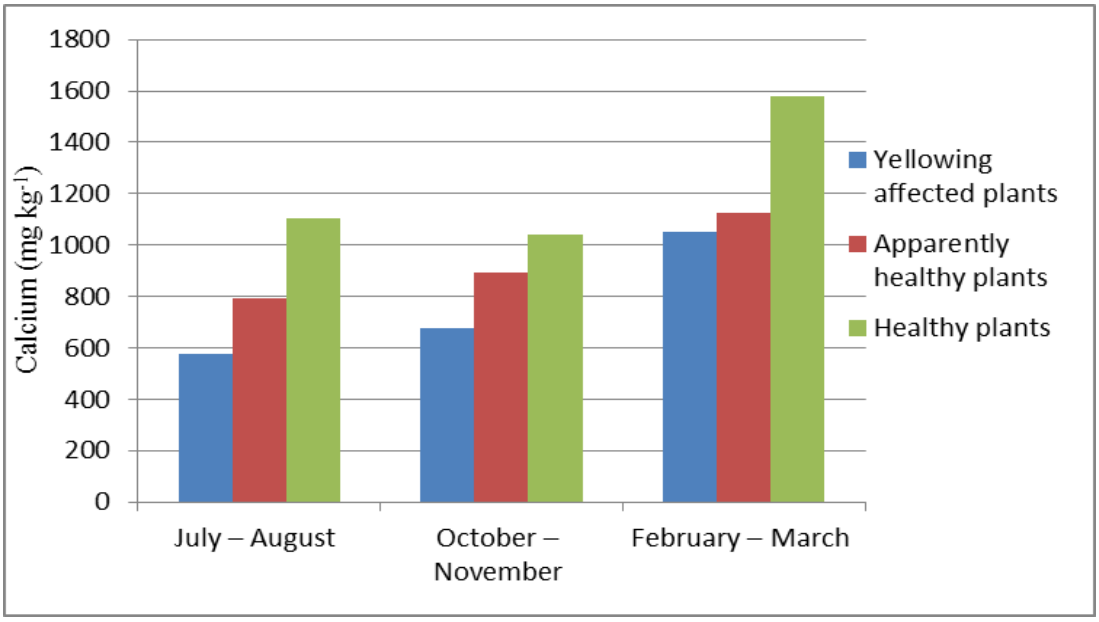


Figure 11. Calcium content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

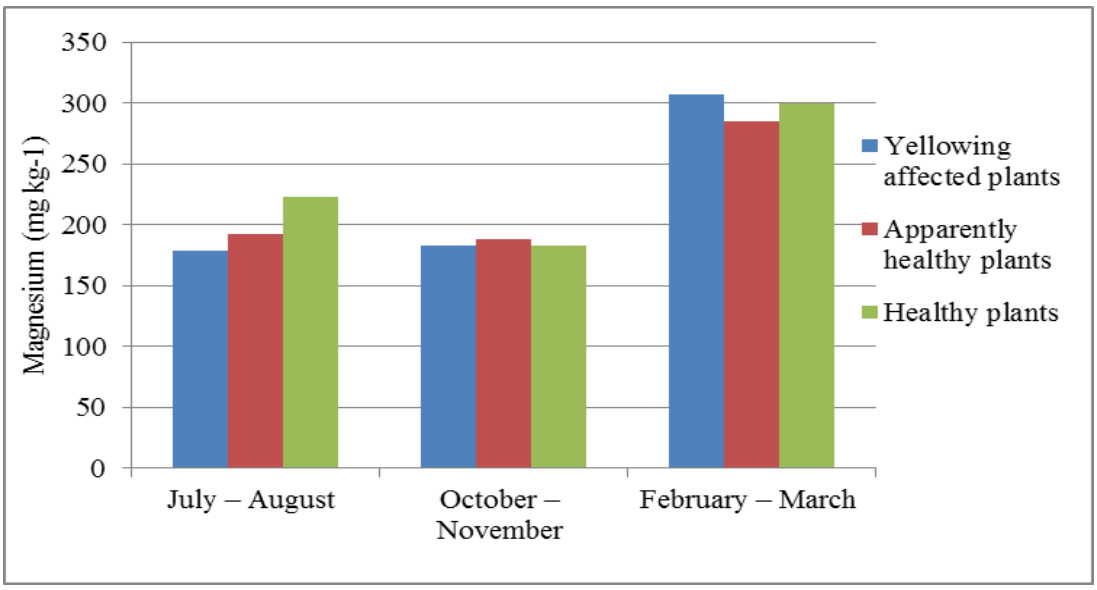


Figure 12. Magnesium content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

5.2.8 Sulphur

Sulphur content in the rhizosphere soils, yellowing affected, apparently healthy and healthy plants ranged between 26.61 mg/kg to 29.31 mg/kg (table 27 and fig 13). There was no significant difference in the sulphur content of rhizosphre soils of the three categories of plant clearly indicating that sulphur content rhizosphere soil does not have a role in causing yellowing in black pepper.

5.2.9 Iron

Iron content in the rhizosphere soil was in the range of 28.52 ppm to 31.99 ppm (table 28). There was no iron deficiency in the rhizosphere soil of healthy, apparently healthy and yellowing affected plants (Hamza *et al*. 2007).

5.2.10 Manganese

Content of Manganese was very high (Hamza *et al*. 2007) in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants (table 29).

5.2.11 Zinc

Zinc content was in the optimum range in apparently healthy and yellowing affected plants (table 30 and fig 14). Whereas, as per the DRIS norms of Hamza *et al*. (2007). The content was high in healthy plants.

5.2.12 Copper

There was no deficiency of copper in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants (table 31). Copper content was in the high range category in all the three categories of plants. Copper content in the black pepper soils are generally reported to be high due to soil application of copper fungicides for the control of phytopthora foot rot disease.

5.2.13 Boron

Boron content was significantly higher in the rhizosphere soil of apparently healthy plants compared to healthy and yellowing affected plants (table 32 and fig 15). Aparna (2017) and Moura *et al*. (2013) pointed out that boron was frequently found in low levels in tropical soils and affected nutrition and productivity of crop plants. CPCRI (2016) reported that Boron deficiency was more in parts of Kerala, Karnataka and West Bengal mainly due to rainy weather, leaching of nutrients through sandy soils.

5.2.14 Aluminium

Aluminium content in the rhizosphere soil of healthy black pepper plants was significantly higher compared to yellowing affected plants (table 33 and fig 16). The result is contradictory to the findings of Sreekumar (2015) who reported high aluminium content in the rhizosphere soil of yellowing affected plants in Wayanad.

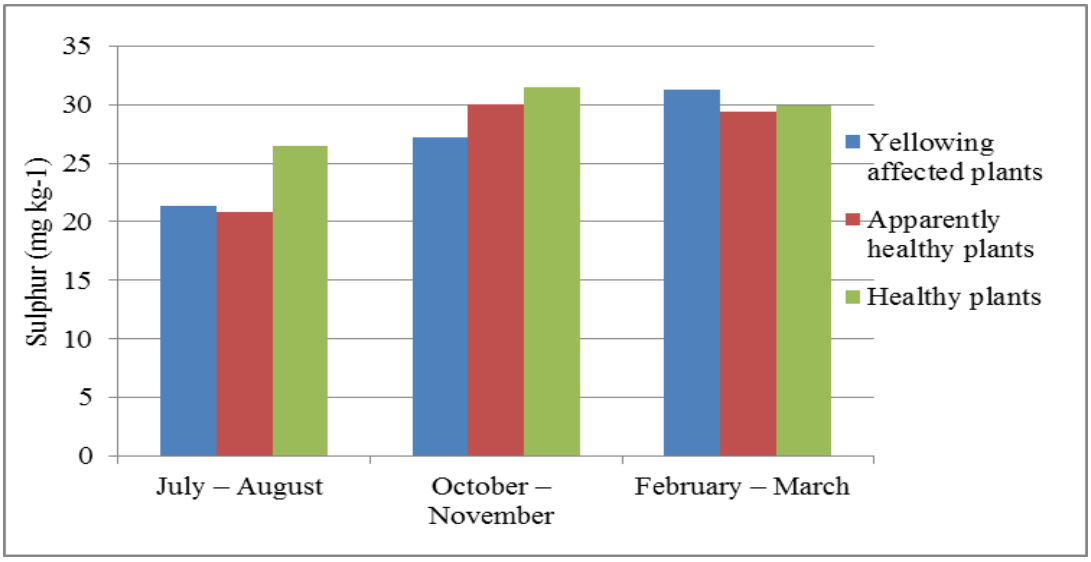


Figure 13. Sulphur content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

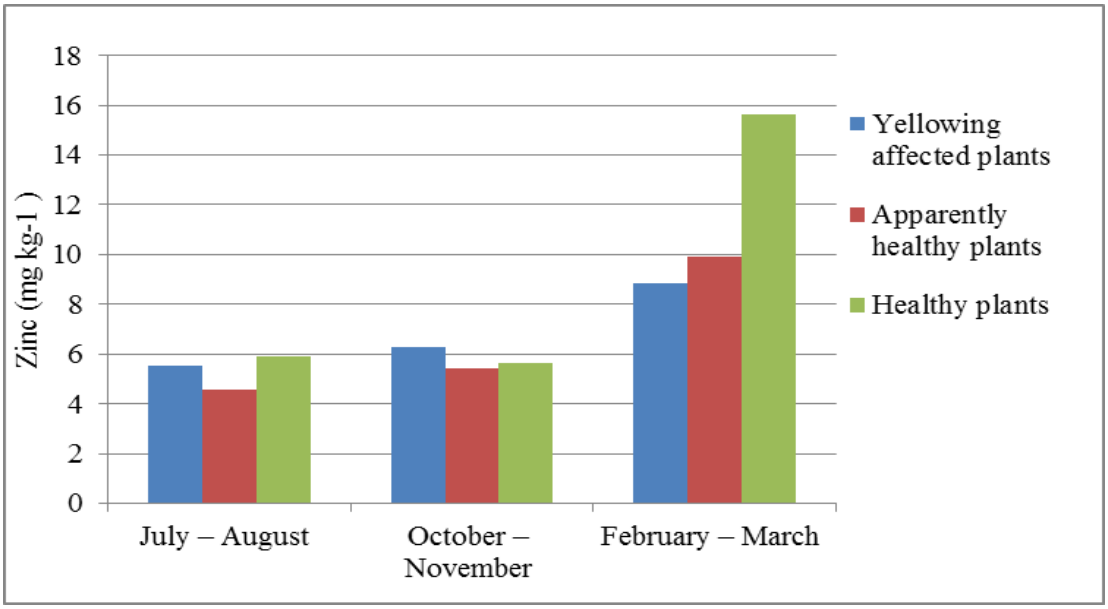


Figure 14. Zinc content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

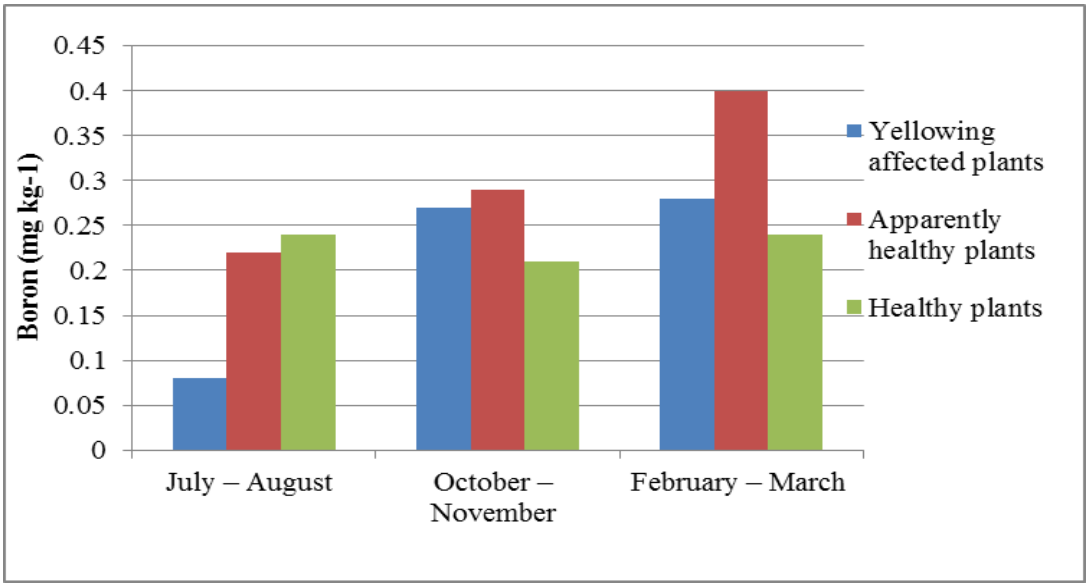


Figure 15. Boron content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

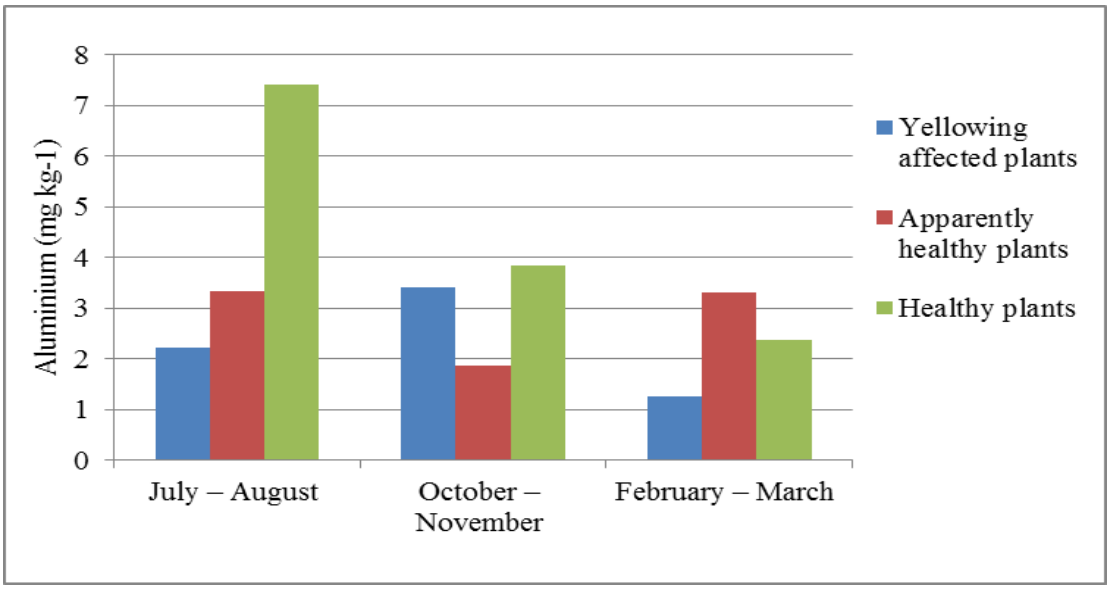


Figure 16. Aluminium content in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

EXPERIMENT-3

5.3 Plant tissue analysis for nutrients

Nitrogen content in the plant samples of apparently healthy plants was lower than critical level (Hamza *et al*. 2007). The content was optimum in healthy plants and deficient in yellowing affected plants (table 34). The results indicated that there was a deficiency of nitrogen in the plant tissues of yellowing affected plants. N deficiency symptoms were reported as pale green colouration of lamina. Subsequently older leaves turned uniformly yellow followed by spreading of the symptoms to younger leaves in severe deficiencies. (Nybe and Nair 1987; De Waard 1969 and Chin *et al*. 1993).

In the case of phosphorus, the content was in the optimum range in yellowing affected and apparently healthy plants as per the DRIS norms of Hamza *et al*. (2007). In the healthy plants phosphorus content was in the high range (table 35). Thus, it can

be observed that there was no deficiency of phosphorus in the leaf tissues of experimental plants.

Considering potassium, the content was in the low range in yellowing affected and apparently healthy plants (table 36). However, potassium content was in the optimum range in healthy plants. However, there was no deficiency of potassium in the three categories of experimental plants, as per the DRIS norms suggested by Hamza *et al* (2007).

Calcium content in the yellowing affected and apparently healthy plants were in the optimum range in the apparently healthy and yellowing affected plants (table 37). However, calcium content was high in the leaf tissues of healthy plants.

There was no deficiency of magnesium also in the leaf tissue of experimental plants. The content of magnesium was high in yellowing affected plants and in the excess range in apparently healthy and healthy plants (table 38).

Even though the overall mean sulphur was in the optimum range in yellowing affected plants (table 39). The content of sulphur during October-November and February-March was in low range in the yellowing affected plants. Sulphur content in the leaf tissue was low in apparently healthy also during October-November and February-March in 2017. However, during 2018 sulphur content in apparently healthy plants was in the optimum range throughout the year. Sulphur deficiency symptoms was reported as chlorosis of younger leaves which turned bright yellow in advanced stage (DeWaard 1969 and Nybe and Nair, 1987).

Iron, manganese, zinc and boron content was in the optimum range in the healthy, apparently healthy and yellowing affected plants in the present study (table 40, 41, 42 and 44). Yellowing of plants in the present study could not be attributed to the deficiency of these elements.

To conclude, in the present study on rhizosphere soil nutrient status, there was no apparent role of soil pH, OC, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B in causing yellowing in the experimental plots. All the nutrient elements were in the optimum or high range. There was no significant difference among yellowing affected, apparently healthy and healthy plants in case of N and S where DRIS norms were not available.

Coming to plant nutrient status, we can see that nitrogen content in the apparently healthy and yellowing affected plants were lower than healthy plants, and nitrogen was deficient in yellowing affected plants. K content was also low in yellowing affected plants during three seasons of study and it was low to optimum in apparently healthy plants. S content also was lower than critical level during October-November and February-March in the yellowing affected plants. All the other nutrient elements were optimum to high in the different categories of plants under study.

5.4 EXPERIMENT-4

5.4.1 Study on association of plant pathogenic organisms with yellowing

From the table 48 and fig 17 it is clear that during July-August, there was no significant difference in the fungal population in the rhizosphere soil of yellowing affected, apparently healthy and healthy plants. During October-November, healthy plants showed significantly high fungal population. During February-March, rhizosphere soils of yellowing affected plants showed significantly high fungal count (24.04×10^3 g^{-1}cfu) when compared with apparently healthy plants (18.06×10^3 g^{-1}cfu) whereas apparently healthy plants were on par with healthy plants.

More over healthy plants showed significantly low population count of Actinomycetes (22.34×10^5 g^{-1}cfu) in the rhizosphere soil, whereas apparently healthy and yellowing affected plants were statistically on par (table 49 and fig 18).

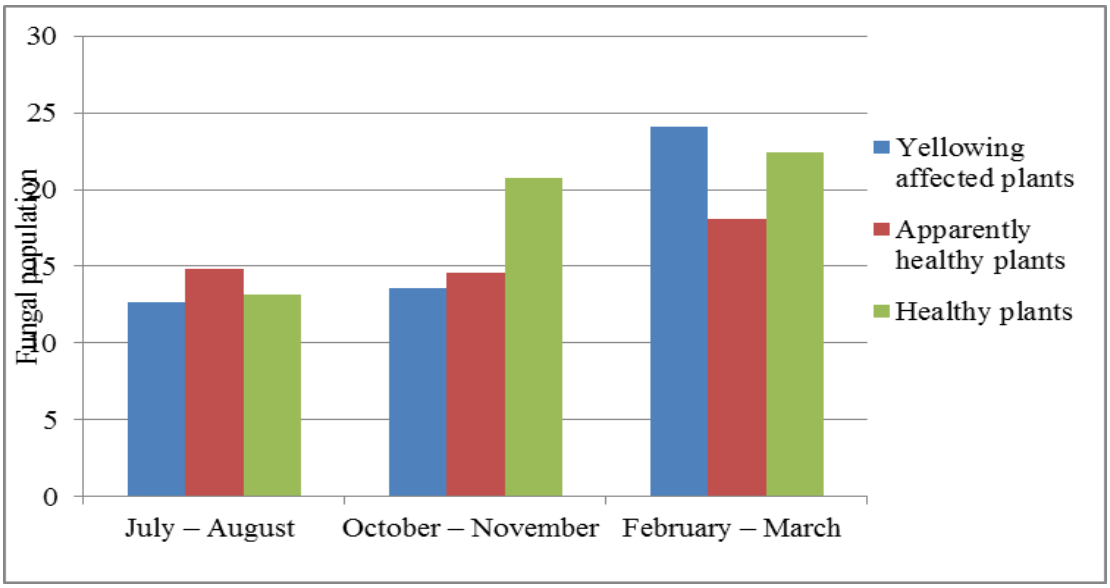


Figure 17. Fungal population in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

Dindal (1990) reported that Actinomycetes prefer to grow in a relatively dry and warm environment with neutral soil pH. Which was found absent in the rhizosphere of healthy plants as the pH was around as (5.66).

Likewise, the yellowing affected plants showed significantly highest population count of bacteria (25.53 $\times 10^{8}$ g^{-1}cfu) followed by apparently healthy plants (table 50 and fig 19), whereas, healthy plants showed significantly lowest count of bacterial population. The variation in population of bacteria in various locations could be due to the variation in soil type, environmental conditions and root exudates. There are several studies which reported that plant roots release variety of organic compounds which favoured the growth of soil bacteria (Neumann and Rom held, 2001)

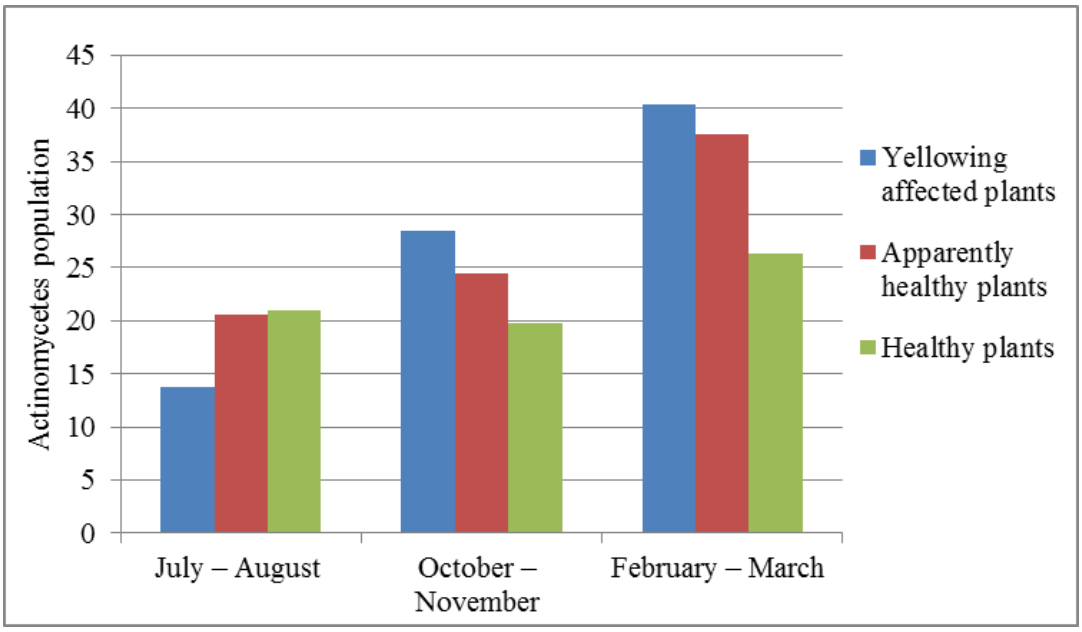


Figure 18. Actinomycetes population in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

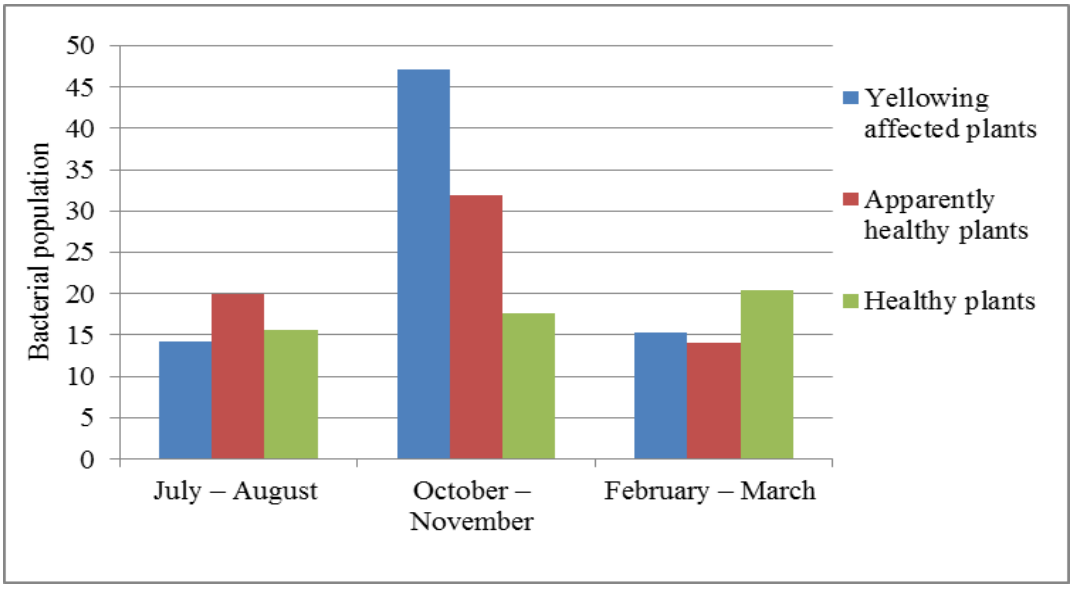


Figure 19. Bacterial population in the rhizosphere soils of healthy, apparently healthy and yellowing affected plants

Shahnazi *et al*. (2012) reported that foliage yellowing of black pepper was due to *Fusarium solani*. Similar findings were reported by Sreekumar (2015) and Bong and Saad (1986). In the present study, no plant pathogenic organism causing yellowing in black pepper could be isolated (*Fusarism*, *Rhizoctonia* or *Phytophthora*) from roots or rhizosphere soil of yellowing affected plants, thus ruling out the possibility of plant pathogenic microbes in causing yellowing in black pepper.

5.5 EXPERIMENT-5

5.5.1 Analysis of rhizosphere soil and root samples for nematode

5.5.1.1 Nematode population in rhizosphere soil

Nematode population was observed during all the three seasons *viz.*, July-August, October-November and February-March. During July-August, the nematode population in the rhizosphere soil was significantly lowest, whereas it was significantly highest during October-November. Nematode population was significantly low in healthy plants during all three seasons, whereas yellowing affected and apparently healthy plants were statistically on par (Table. 51and fig 20). Mohandas and Ramana (1988) reported that lowest population of *Radopholus similis* was observed during April-May which gradually increased reaching a peak during September-October. In the present study also it was observed that nematode population was highest during October-November. Mohandas and Ramana (1988) opined that the population of nematodes reached the lowest level during April/May when soil temperature was high and soil moisture was low. Then the population started slowly rebuilding and reached a peak during September-October. However, Thuy *et al.* (2012) reported that root population densities of *M. incognita* decreased towards the end of dry season and remained low during rainy season. In the present study nematode population was found lowest during July-August. Thuy *et al.* (2012) also reported that in sites with short rainy season root galling was highest in the

middle of dry season. In sites with longest rainy season highest percentage root galling was observed at the end of the rainy season.

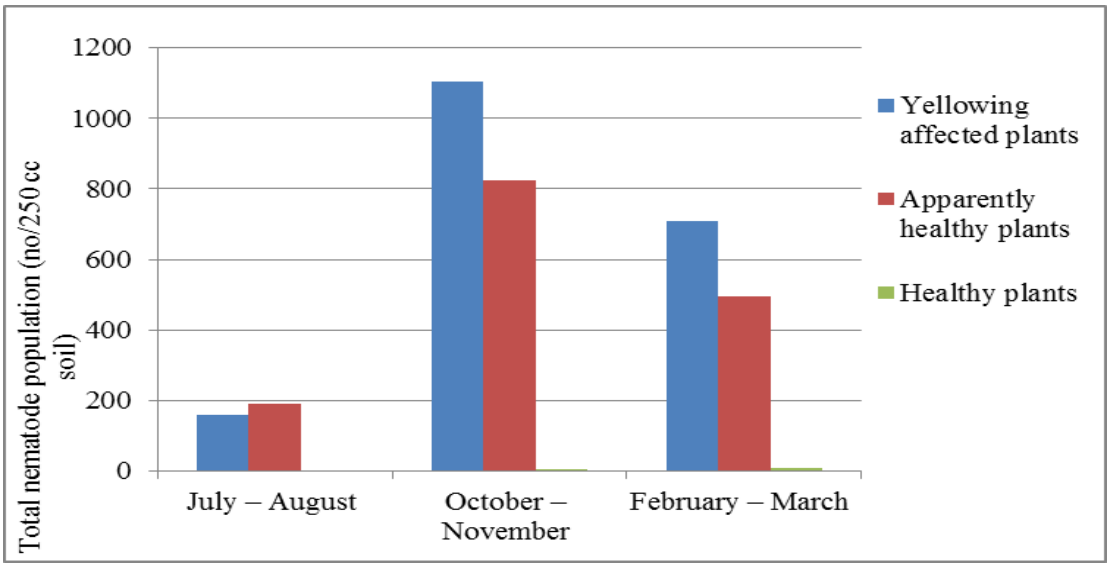


Figure 20. Total nematode population in rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

5.5.1.2 Seasonal variation in the nematode population in roots

As in the case of nematode population in the soil, total nematode population of roots also was significantly highest during October-November and population was significantly lowest during July-August compared to October-November and February-March (table 58).

Number of galls in the roots was also significantly highest during October-November and lowest during July-August (table 59).

Population of *M. incognita* in the roots was significantly highest during October-November and lowest during July-August (table 60).

Population of burrowing nematode *Radopholus similis* in the roots was also lowest during July-August and population was statistically on par during October-

November and February-March (table 61). Rashid *et al*. (2017) reported that root-knot nematode population in roots of pepper reached maximum during April-May and minimum during December and January. A low soil temperature coupled with adequate soil moisture availability of fresh tender roots helped in the buildup of its population during September- October. The nematode was detectable throughout the year. Various factors like, rainfall and temperature influenced nematode populations.

In India, the predominant nematode species associated with black pepper plants reported were *Meloidogyne* sp., *R. similis*, *Trophotylenchus piperis*, *Helicotylenchus* sp. and *R. reniformis, Criconemoides, Xipnema, Hoplolamus, Pratylenchus* (Ramana and Mohandas, 1987).

Different plant parasitic nematodes obtained from the surveyed area in the present study were *Meloidogyne incognita, Radopholus similis, Pratylenchus* sp; *Helicotylenchus* sp., *Dorylamid* sp. and *Trophotylenchulus* sp. *Dorylamid* sp. was reported for the first time in black pepper.

Among the plant parasitic nematodes associated with black pepper in the present investigation, *M. incognita* and *R. similis* were most predominant ones. Their numerical strength compared to other species of plant parasitic nematodes that were encountered in the black pepper rhizosphere was high. Thus, clearly proving their role as elicitants of yellowing in pepper.

5.5.1.3 Meloidogyne incognita

The *Meloidogyne incognita* population was significantly low in the rhizosphere soils of healthy plants and was on par in yellowing affected plants and apparently healthy plants (table 52 and fig 21). The first record of root–knot nematode infestation on pepper was reported from Cochin – China presently a part of Vietnam by Delacroix (1902). Almost during the same period, (Ridley 1912) observed root-knot nematode infestation on pepper in Wayanad, Kerala, India.

Ayyar (1926) reported the wide spread occurrence of root knot nematodes in Wayanad. Root knot nematode infestation were also reported from many pepper growing countries like Malaysia (Holliday and Mowat 1963; Kueh, 1975; Ting, 1975; Razak, 1981), Indonesia (Ichinohe, 1976, Bridge, 1978), Brazil (Sharma and Loof 1974; Ichinohe, 1975); Thailand (Sher *et al*., 1969), Fiji (Swaine 1971), Guayana (Biessar, 1969) and Srilanka (Lamberti *et al*. 1983). Pepper plants infested with root knot nematodes generally exhibited foliar yellowing, poor growth and gradual decline in health and vigour. Sometimes leaves of infested vines showed dense yellowing of inter veinal areas making the leaf veins quite prominent with deep green colour (Ramana, 1994).

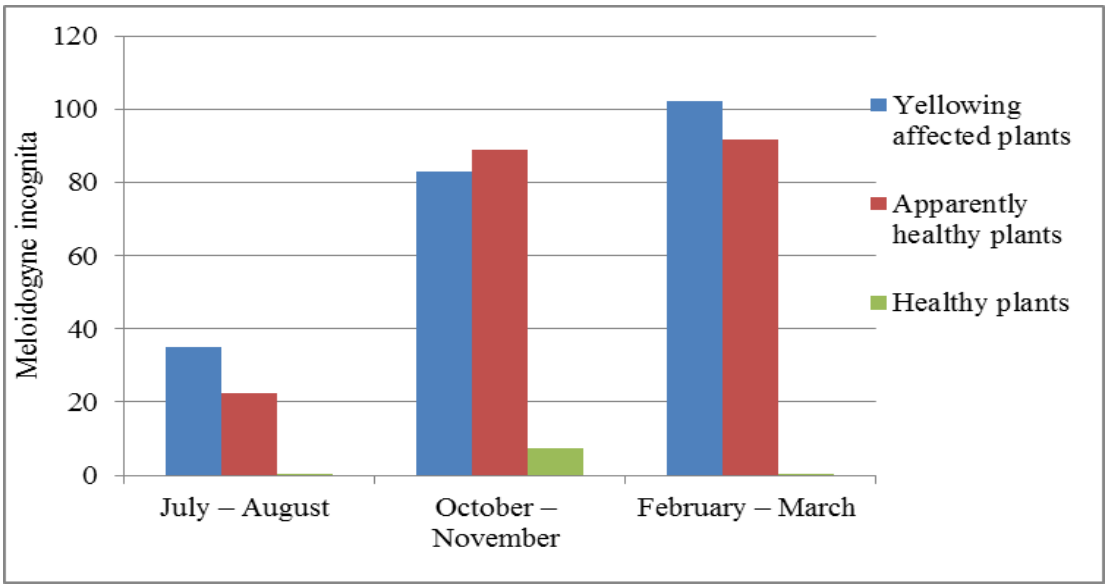


Figure 21. *Meloidogyne incognita* population in rhizosphere soils of healthy, apparently healthy and yellowing affected plants

5.5.1.4. Radopholus similis

The population of *Radopholus similis* was significantly lowest in the rhizosphere soils of healthy plants when compared with yellowing affected and apparently healthy plants (table 53 and fig 22). During February-March, in the

rhizosphere soils of yellowing affected plants significantly higher number of *Radopholus similis* population was observed compared to apparently healthy and healthy plants. The major nematodes associated with yellowing affected black pepper gardens were *M. incognita* and *Radopholus similis.* Ramana *et al.* (1987) also reported high population *R. similis* (over 250 /g of roots) with slow wilt affected pepper vines.

Plate 16. Gall formation in nematode infested black pepper roots

5.5.1.5 Damage caused due to nematode infestation

Koshy *et al*. (2005) reported that *R. similis* caused a uniform pale yellow or whitish discoloration while *Meloidogyne* sp caused dense yellowish discoloration of the interveinal areas making leaf veins quite prominent with a deep green colour.

Wahid and Sitepu (1987) reported that the symptoms of the disease were foliar yellowing and leaf fall in both young and older plants. The yellowing of leaf started from the bottom of the plant and spread to the top, covering the whole plant at later stages of the disease. They were also of the opinion that the disease was mainly due to plant parasitic nematodes, *R. similis*, *M. incognita* and the fungus *Fusarium* sp. combined with agronomic disorders. Mohandas and Ramana (1987) reported that slow decline was reported as a debilitating disease over a period of time. The above ground symptoms of the disease were yellows, defoliation, die-back, loss in vigourand productivity, leading to slow death. On roots, nematode infestation resulted in the formation of galls due to root-knot nematodes, necrotic lesions and rotting caused by *R. similis* resulted in total loss of feeder roots. Infested plants sometimes recovered with the onset of monsoon when the plants put forth new roots and leaves. However, the plants succumbed to the disease as the `root generation could not compensate the root loss due to nematode damage.

In Indonesia, burrowing nematode *Radopholus similis* was reported to destroy black pepper fields (Sitepu and Mustika, 2000). In Vietnam, 49 nematode species were found in all most all pepper producing regions. Root knot nematode (*Meloidogyne* sp) was the most serious.

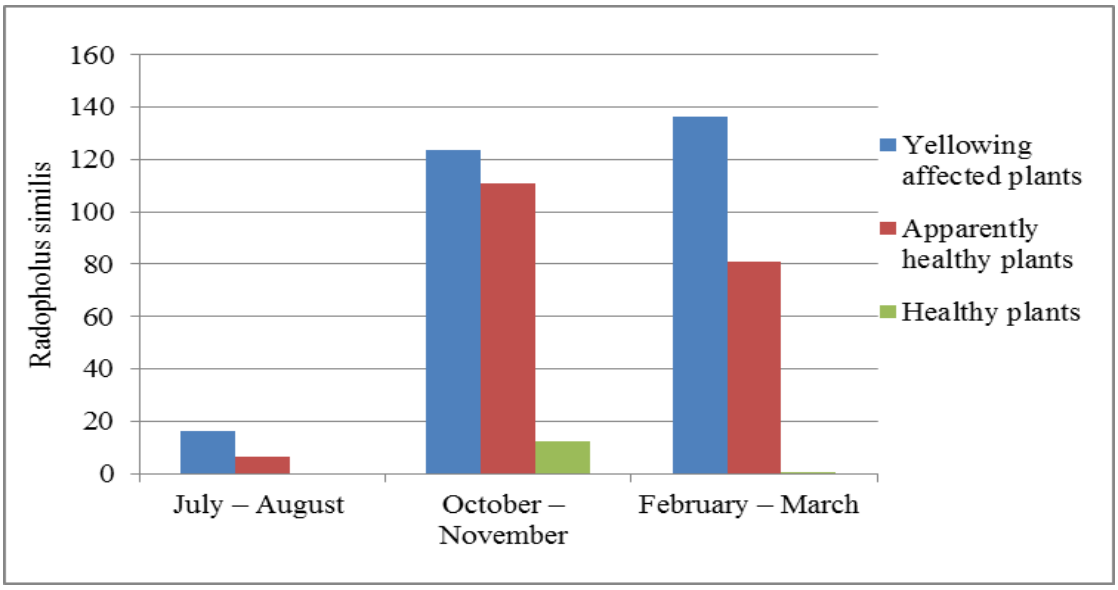


Figure 22. *Radopholus similis* population in rhizosphere soils of healthy, apparently healthy and yellowing affected plants.

In their reviews of plant-parasitic nematodes associated with black pepper plants, Sundararaju *et al*. (1979) listed 48 nematode species belonging to 29 genera, Ramana and Eapen (1998) listed 54 nematode species belonging to 30 genera while Koshy *et al*. (2005) listed 28 nematode species belonging to 17 genera. In India, the predominant nematode species associated with black pepper plants were *Meloidogyne* spp., *R. similis*, *Trophotylenchus piperis*, *Helicotylenchus* sp. and *R. reniformis* (Ramana and Mohandas 1987).

A definite positive correlation of plant parasitic nematode population in soil and yellowing was observed in the present study as given in table 65. Certain physiological changes were also observed in plants infested with *M. incognita*, like reduction in absorption of P, K, Zn, Mn, Cu, Ca and Mg deficiency as observed by Ferraz *et al*. (1988).

5.6 EXPERIMENT VI

5.6.1 Identification of root mealy bugs

Roots and soil samples were analysed during three seasons for two years in healthy, apparently healthy and yellowing affected plants. Examination of roots of experimental plants and rhizosphere soil did not show the presence of root mealy bugs or any other insect, which can cause damage to root or yellowing in the plant. The possibility of any insect including root mealy bug involved in causing yellowing of black pepper in the experimental area was ruled out.

5.7 EXPERIMENT VII

5.7.1 Correlation with weather variables

5.7.1.1 Correlation of yellowing with weather variables

There was significant positive correlation of yellowing with maximum temperature. However minimum temperature showed significant negative correlation with yellowing. Relative humidity and rainfall also were significantly and negatively correlated with yellowing (table 64). (Sushna and Ajith, 2017) reported that rainfall and relative humidity positively affected the leaf and root production of black pepper varieties. Whereas pepper production declined due to increased temperature and bright sunshine hours. Vijayakumar *et al*. (1985) reported that even with favourable soil moisture conditions, the leaves of black pepper plants exposed to direct solar radiation developed symptoms yellowing, followed by formation of necrotic patches.

5.7.1.2 Correlation of yellowing and soil nutrients

The correlation of different rhizosphere soil characters with yellowing is given in table 65. It can be seen from the table that the soil pH, EC and Organic Carbon were significantly and positively correlated with yellowing. There was no significant correlation of N, P, Mg, S, Fe, Mn, Zn, Cu and B content of rhizosphere

soil with yellowing in black pepper. K, Ca and Al content of rhizosphere soils showed negative significant correlation yellowing.

5.7.1.3 Correlation of yellowing and tissue nutrients

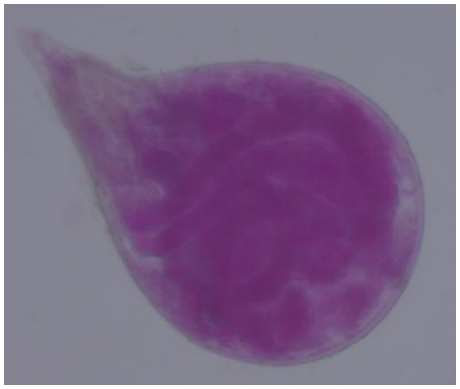
Tissue analysis of yellowing affected, apparently healthy and healthy plants showed negative correlation of yellowing with N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Al content in plant samples. (table 66).

5.7.1.4 Correlation of soil nematode population with weather variables

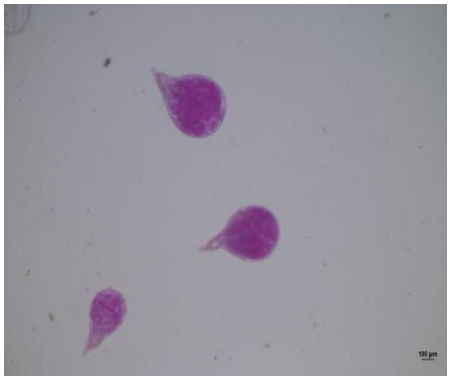
Significant positive correlation was noticed with the soil nematode population and yellowing. The soil nematode population showed significant negative correlation with minimum temperature, relative humidity and rainfall. However Mohandas and Ramana, (1988) reported that rainfall had an indirect effect on the increase in the nematode numbers by reducing the soil temperature and by inducing the host plant to put forth new roots which provided more feeding sites for nematodes. Rainfall also increased soil moisture which helped the nematodes to migrate from infested roots to healthy roots. They also reported that the rainfall and number of rainy days in a month was significantly correlated with the population density of *R. similis* during the subsequent months. Thuy *et al.* (2012) reported that during rainy season nematode population was low due to excess of water resulting in an oxygen deficit and many nematode species succumbed to these conditions. This observation is in agreement with the present study. In contrast, too little water inhibited the hatching of eggs and limited the movement of nematodes in the soil.

All other factors (soil chemical characters, microflora, soil borne pathogens *etc*.) showed no association or negative correlation with yellowing. Nematodes were identified to be the primary cause associated with yellowing in black pepper in the present study. Nematodes are slowly debilitating pathogen resulting in yellowing.

Plate 17. Stages of infestation of *M. incognita* on roots of yellowing affected black pepper



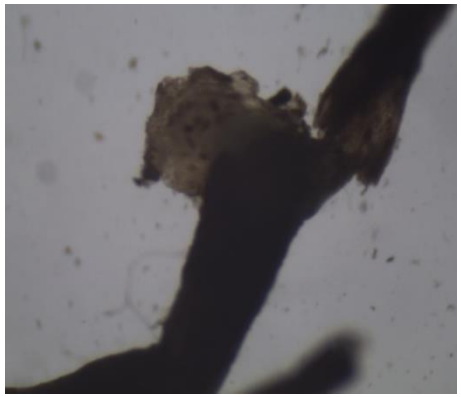
Female adult



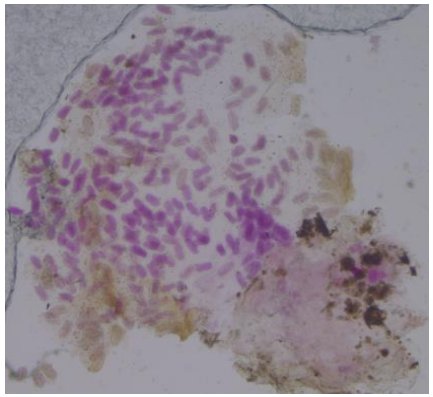
Adult females from single gall

Egg mass on root lesion

Root gall with egg mass



Single egg sac



Eggs after staining

Plate 18. Increase in severity of yellowing during the period under study

Early symptoms

Advanced stage of yellowing

Death of affected plant

` The affected plants recovered to some extend in the next rainy season due to suppression of nematode population in soil and rooting and growth of black pepper during favorable weather.

In the present study, analysis of different factors like soil nutrients, plant pathogenic micro-organisms, soil borne insects, nematodes *etc*. gave a clear indication of role of nematodes in causing yellowing in black pepper in the experimental plots in Thrissur district. Even though the soil nutrient status in the rhizosphere soil of experimental plants was satisfactory, absorption of nutrients was severely affected due to damage of roots caused by nematodes. The yellowing expressed must be due to multiple factors and combined effect of nematode infestation and multiple nutrient deficiencies in plant tissue.

In the month October-November average monthly rainfall was 241.7 mm and the average monthly maximum and minimum temperature were 31.7^{o} and 22.3^{o} C. Relative humidity noticed was 81 per cent. The population of nematodes in the rhizosphere soil and root was more during October-November. High population of nematode in the roots and soil indicated that the climatic condition prevailed in October-November was favourable for population buildup of nematodes. It might be a serious problem destroying the feeder roots and as results the plant exhibited, yellowing and discoloration in upper canopy of infected pepper plants.

Nematode species have shown a decline in population during rainy season. According to our study, the rhizosphere soils had optimum range of nutrients in soil but the root system was destroyed due to heavy incidence of endoparasitic nematodes. The nutrient uptake was very poor in plants in which the roots were severely infested by nematode. However, these plants recovered under ideal environmental conditions. Moreover, plants started to form new feeder roots during ideal season, because plants again turned green from yellow. When the nematodes completed their life cycle in particular plant, it moved to another plant. It was seen

that apparently healthy plants turned to yellowing affected plants under unfavorable condition.

The population of plant parasitic nematodes in the soil was alarming and an immediate step to control nematode population is warranted. Confirmation of causal organism (nematodes) in causing yellowing is required by cross inoculating pepper plants in nursery bags. Formulations of proper prophylactic/control measures are also warranted.

Summary

6. SUMMARY

The research project entitled "Investigations on yellowing of black pepper (*Piper nigrum* L.)" was conducted for three years from July 2016 to February 2019. After foot rot disease, the major constraint in black pepper cultivation is the incidence of yellowing where the production was found to decline year after year. Yellowing was reported to be a complex disease caused by many factors like poor soil health, improper land management practices and changes in climatic factors and biotic and abiotic stresses. The present study was undertaken to find the role of soil and plant nutrients, plant pathogens, insects, nematodes and climatic factors, if any in causing yellowing of black pepper in Thrissur district of Kerala.

6.1 STUDY ON THE INITIATION AND DEVELOPMENT OF YELLOWING

- Symptomatological studies on yellowing showed that per cent severity of yellowing was significantly higher during October-November and was on par during summer. During July-August, yellowing was significantly low.

- Yellowing was observed either at the top of pepper column or (and) the bottom of the column. Sometimes yellowing appeared in a group of leaves together. In some aged plants older leaves at terminal portion of plant were yellow. In some leaves the base of lamina near petiole was yellow. This yellowing faded to tip of lamina and margins.

- The symptoms of yellowing were highly varying from plant to plant. There was no definite pattern in the development and spread of the disease.

- In case of mild yellowing, there was recovery during July-August. Yellowing gradually increased and severely affected plants failed to recover. There was no flushing or flowering in plants. Such plants died in 2-3 years.

- All varieties studied were found to be susceptible to yellowing.

- Yield and yield contributing characters in healthy, apparently healthy and yellowing affected black pepper plants showed that all the characters of healthy plants showed a higher positive value compared to yellowing affected plants. In the case of yield per plant, there was significant reduction in yield in yellowing affected plant compared to healthy in all varieties except in the variety Vijay.

6.2 ANALYSIS OF RHIZOSPHERE SOIL CHARACTER AND THEIR ASSOCIATION WITH YELLOWING

- It was observed that rhizosphere soil of healthy plants showed significantly lower pH compared to yellowing affected plants. Low pH of the rhizosphere soil could not be considered as the cause of yellowing in the present study

- Organic carbon content of the experimental plots were lower than critical values. Organic carbon content was found to be significantly high in the rhizosphere soil of yellowing affected plants which clearly showed that yellowing was not due to a deficiency in organic carbon content in the rhizosphere soil.

- Nitrogen content in the rhizosphere soil did not have a direct role in causing yellowing in the experimental plots of the present study.

- Phosphorus content of rhizosphere soil of experimental plants was above 96 ppm which was reported to be high. Phosphorus content in the rhizosphere soil of healthy and yellowing affected plants were statistically on par.

- In the present study apparently healthy and yellowing affected plants showed K content with in optimum to high range. Healthy plants showed potassium content in the high range throughout the period of study

- There was no deficiency of calcium, magnesium, iron and zinc in the rhizosphere soils of experimental plants. Manganese, zinc, copper and aluminium content were higher than optimum level in the healthy plants.
- In case of sulphur, rhizosphere soils of healthy plants showed significantly higher S content compared to apparently healthy and yellowing affected plants.
- Copper content was high in all the three categories of plants.
- Boron content was significantly higher in the rhizosphere soil of apparently healthy plants compared to healthy and yellowing affected plants
- Aluminium content in the healthy black pepper plants was significantly higher compared to yellowing affected plants
- In the present study on rhizosphere soil nutrient status showed that there was no apparent role of soil pH, OC, N, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B in causing yellowing in the experimental plants. All the nutrient elements were in the optimum or high range.

6.3 PLANT TISSUE ANALYSIS FOR NUTRIENTS

- Unlike soil nutrient status, tissue analysis of healthy, apparently healthy and yellowing affected plants showed that there was significantly higher content of P, Ca, Mg, Fe, Mn, Zn, Cu and B in healthy plants compared to yellowing affected plants. A negative correlation was observed between yellowing and plant nutrient status.
- These results clearly showed that even though there was high level of nutrients in soil, the absorption of nutrients was low in yellowing affected plants.

- Analysis of nutrient status in the plant tissues of yellowing affected, apparently healthy and healthy plants based on DRIS norms suggested by Hamza *et al.* (2007) indicated that there was deficiency of N in the yellowing affected plants, K content and S content was also low in yellowing affected plants. Other nutrients were not deficient in the yellowing affected plants.

6.4 STUDY ON ASSOCIATION OF PLANT PATHOGENIC ORGANISMS WITH YELLOWING

- During October-November, healthy plants showed significantly high fungal population. The mean value of fungal population was highest in rhizosphere soils of healthy plants.

- Healthy plants showed significantly low population count of actinomycetes in the rhizosphere soil, whereas apparently healthy and yellowing affected plants were statistically on par.

- Yellowing affected plants showed significantly highest population count of bacteria followed by apparently healthy plants. Healthy plants showed significantly lowest count of bacterial population.

- No plant pathogenic organism causing yellowing in black pepper could be isolated (*Fusarism*, *Rhizoctonia* and *Phytopthora*) from roots or rhizosphere soil of yellowing affected plants. Thus, ruling out the possibility of plant pathogenic microbes in causing yellowing in black pepper in the present study.

6.5 NEMATODE POPULATION IN RHIZOSPHERE SOILS OF BLACK PEPPER

- There was heavy incidence of plant parasitic nematodes in the roots and rhizosphere soil of yellowing affected and apparently healthy plants. Major species present were *Meloidogyne incognita* and *Radopholus similis*.

- Healthy plants recorded significantly low level of nematodes compared to apparently healthy and yellowing affected plants

- During October-November the nematode population in rhizosphere soils was significantly highest compared to February-March and July-August.

- The population of *M. incognita* was significantly lowest in the rhizosphere soils of healthy plants during July-August compared to October-November and February-March. However, apparently healthy and yellowing affected plants were statistically on par during all the three seasons.

- Association of *Dorylamid* sp. with black pepper was reported for first time.

- *Pratylenchus* and *Dorylamid* species were present in apparently healthy and yellowing affected plants but absent in rhizosphere soil of healthy plants, Similarly, population of *Helicotylenchus* and *Trophotylenchulus* were significantly low in healthy plants compared to yellowing affected.

- A definite positive correlation of plant parasitic nematode population in soil and yellowing was observed in the present study

6.6 ROLE OF ROOT MEALY BUGS IN CAUSING YELLOWING

- Examination of roots and rhizosphere soil of experimental plants did not show the presence of root mealy bugs or any other insect, which can cause damage to root or yellowing in the plant.

6.7 CORRELATION OF YELLOWING WITH WEATHER VARIABLES

- There was significant positive correlation of yellowing with maximum temperature. However minimum temperature showed significant negative correlation with yellowing. Relative humidity and rainfall also were significantly and negatively correlated with yellowing

6.8 CORRELATION OF YELLOWING WITH SOIL AND PLANT NUTRIENTS

- It can be seen from the table that the soil pH, EC and organic carbon were significantly and positively correlated with yellowing. There was no significant correlation of N, P, Mg, S, Fe, Mn, Zn, Cu and B content of rhizosphere soil with yellowing in black pepper. K, Ca and Al content of rhizosphere soils showed significant negative correlation with yellowing

- Tissue analysis of yellowing affected, apparently healthy and healthy plants showed negative correlation of yellowing with N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B and Al content in plant samples.

Conclusion

In the present study on analysis of different factors like soil nutrients, plant pathogenic micro-organisms, soil borne insects, nematodes *etc.*, in causing yellowing has given a clear indication on role of nematodes in the experimental plots in Thrissur district. Even though the soil nutrient status in the rhizosphere soil of experimental plants was satisfactory, absorption of nutrients was low due to damage of roots caused by nematodes. There was deficiency of nitrogen and low potassium and sulphur levels in the leaves of yellowing affected plants. The yellowing expressed must be due to multiple factors and combined effect of multiple nutrient deficiencies in plant tissue and damage due to nematodes.

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APPENDICES

Crop management practices followed by farmers in the selected fields

Location-1

(Black pepper research unit, Dept. of Plantation Crops & Spices, College of Horticulture)

- FYM/Compost @ 5-10 kg/plant
- Lime @ 500 g /plant in alternate years
- Factamfos - 250 g/plant
- Muriate of Potash @ 100 g/plant
- Drenching Copper oxychloride 3 g/l @ 5-6 liters plant during June/July
- Spraying Copper hydroxide 1.5 g/ plant
- Use of bio control agents pseudomonas/ Trichoderma

Location-2

(Gokhale Block, Dept. of Plantation Crops & Spices, College of Horticulture)

- Urea (46 % N) @ 250 g per plant applied as a source of nitrogen
- Muriate of Potash 60% @ 100 g/plant was applied as a source of potassium
- Bone meal (30% phosphorus) @ 250 g per plant as a phosphatic fertilizers
- Neem cake @ 500 g per plant and Lime application @ 100 g per plant during post monsoon season
- Biological control *viz.* Pseudomonas/ *Trichoderma* along FYM/Compost used for yellowing affected plants

- Kocide (Copper oxychloride 53.8%) fungicide drenching and foliar application for prevent diseases
- Insecticide Marshal (Carbosulfan 255 EC) drenching to prevent the soil borne insects.

Location-3

(Ameena. K. K, Chirakakkode, Farmers field)

- Provided deep drainage channels so as to keep the water below the root zone during monsoon and undertake periodic cleaning of drainage channels to facilitate better drainage to prevent *Phytopthora* disease
- 200 g/plant/year lime applied in the basin of the plant before monsoon
- FYM applied at the rate of 10 Kg/plant in two split doses during pre-monsoon and post monsoon.

Location-4

(Dr. Anitha. M, University Nagar, Mannuthy, Farmers field)

- Provided deep drainage channels so as to keep the water below the root zone during monsoon and undertake periodic cleaning of drainage channels to facilitate better drainage to prevent *Phytopthora* disease
- 200 g/plant/year lime applied in the basin of the plant before monsoon
- FYM applied at the rate of 10 Kg/plant in two split doses during pre-monsoon and post monsoon.
- Kocide (Copper oxychloride 53.8%) fungicide drenching and foliar application to prevent yellowing and fungal diseases
- Maintain field free from weeds & pest and diseases

Location-5

(N. Bose, Chelakkara, Farmers field)

- Provided deep drainage channels so as to keep the water below the root zone during monsoon and undertake periodic cleaning of drainage channels to facilitate better drainage to prevent *Phytopthora* disease
- 200 g/plant/year lime applied in the basin of the plant before monsoon
- FYM applied at the rate of 10 Kg/plant at two split doses during pre-monsoon and post monsoon.
- Collected medicinal plants wastage from Oushadi after extraction, mixed along with FYM and applied to the pepper plants.

Location-6

(Joseph. P, Vaniyampara, Farmers field)

- Provided deep drainage channels so as to keep the water below the root zone during monsoon and periodic cleaning of drainage channels to facilitate better drainage to prevent *Phytopthora* disease.
- 200 g/plant/year lime applied in the basin of the plant before monsoon
- FYM applied at the rate of 10 Kg/plant at two split doses during pre-monsoon and post monsoon.
- Inorganic fertilizer application was done @ 100:40: 200g NPK/plant/year in two split doses in February and September
- Biological controls *viz.* Pseudomonas/ *Trichoderma* along FYM/Compost used for yellowing affected plants and additionally goat manure applied as a fertilizer.
- Routine fungicide and insecticide application were carried out.

"INVESTIGATIONS ON YELLOWING OF BLACK PEPPER (*Piper nigrum* L.)"

By

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(2016-22-002)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES

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2019

ABSTRACT

Black pepper, popularly known as King of Spices or Black gold is one of the important and earliest known spices produced and exported from India. After foot rot disease, the major constraint in black pepper cultivation is the incidence of yellowing, leading to decline in production year after year. Yellowing is reported to be caused by many abiotic and biotic factors.

Symptomatological studies on yellowing showed that per cent severity of yellowing was significantly higher during October-November and was on par during summer. During July-August, yellowing was significantly low. There was no definite pattern in the development and spread of the disease.

In case of mild yellowing, there was recovery during July-August. Yellowing gradually increased and severely affected plants failed to recover. There was no flushing or flowering in plants. Such plants died in 2-3 years. All varieties studied were found to be susceptible to yellowing. Yellowing was observed either at the top of pepper column or (and) the bottom of the column. Sometimes yellowing appeared in a group of leaves together. In some aged plants older leaves at terminal portion of plant were yellow. In some leaves the base of lamina near petiole was yellow. This yellowing faded to tip of lamina and margins.

Yield and yield contributing characters in healthy, apparently healthy and yellowing affected black pepper plants showed that all the characters of healthy plants showed a higher positive value compared to yellowing affected plants. In the case of yield per plant, there was significant reduction in yield in yellowing affected plants compared to healthy in all varieties except Vijay.

In the study on rhizosphere soil nutrient status there was no apparent role of soil pH, OC, N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu and B in causing yellowing in the experimental plots in the present study. All the nutrient elements were in the optimum

or high range. There was no significant difference among yellowing affected, apparently healthy and healthy plants in case of N and S where DRIS norms were not available.

Among the rhizosphere soil parameters, pH, EC and organic carbon were significantly and positively correlated with yellowing. There was no significant correlation of N, P, Mg, S, Fe, Mn, Zn, Cu and B content of rhizosphere soil with yellowing in black pepper. K, Ca and Al content of rhizosphere soils showed significant negative correlation with yellowing.

Analysis of nutrient status in the plant tissues of yellowing affected, apparently healthy and healthy plants based on DRIS norms suggested by Hamza *et al.* (2007) indicated that there was deficiency of N in the yellowing affected plants, K content and S content was also low in yellowing affected plants, all other nutrients were not deficient in the yellowing affected plants.

Study on soil micro flora (cfu g^{-1}) showed that mean value of fungal population was significantly highest (18.78 $\times 10^{3}$cfu g^{-1}) in rhizosphere soils of healthy plants compared to apparently healthy and yellowing affected plants. Healthy plants showed significantly low (27.55 $\times 10^{3}$cfu g^{-1}**)** population count of actinomycetes in the rhizosphere soil, whereas apparently healthy and yellowing affected plants were statistically on par. Yellowing affected plants showed significantly highest population count of bacteria (25.53 $\times 10^{8}$cfu g^{-1}) followed by apparently healthy. Healthy plants showed significantly lowest bacterial population. No soil borne pathogens including *Phytophthora*, *Rhizoctonia* or *Fusarium* was found associated with yellowing

Nematode population in the rhizosphere soils of healthy plants was significantly lowest compared to apparently healthy and yellowing affected plants. Yellowing affected plants showed significantly highest population of *Meloidogyne incognita*, *Radopholus similis*, *Pratylenchus* sp, *Helicotylenchus* sp, *Dorylaimid* sp,

and Trophotylenchulus sp, in the rhizosphere soil when compared with apparently healthy and healthy plants. In case of root, number of galls on the roots was significantly highest in yellowing affected plants. Considering different categories of nematodes, *Meloidogyne incognita* followed by *Radopholus similis* were predominant in the roots of yellowing affected plants. In roots as well as rhizospere soil, significantly highest population of nematodes was observed during October – November followed by February – March.

Examination of roots of experimental plants and rhizosphere soil did not show the presence of root mealy bugs or any other insect, which can cause damage to root or yellowing in the plant.

There was significant positive correlation of yellowing with maximum temperature. However minimum temperature showed significant negative correlation with yellowing. Relative humidity and rainfall also were significantly and negatively correlated with yellowing.

A definite association of plant parasitic nematodes was seen in the plants as well as rhizosphere soils of yellowing affected plants in the present study. *Meloidogyne incognita* followed by *Radopholous similis* population was significantly highest in yellowing affected plants indicating their role clearly in causing yellowing.

In the present study on the analysis of different factors like soil nutrients, plant pathogenic micro-organisms, soil borne insects, nematodes *etc*, in causing yellowing gives a clear indication of role of nematodes in causing yellowing in black pepper in the experimental plot in Thrissur district. Even though the nutrient status in the rhizosphere soil of experimental plants was satisfactory, absorption of nutrients was low due to damage of roots caused by nematodes. There was deficiency of nitrogen and low potassium and sulphur levels in the leaves of yellowing affected plants. The yellowing expressed must be due to multiple factors and combined effect of multiple nutrient deficiencies in plant tissue and damage due to nematodes.