CHAPTER-6

Summary
CHAPTER-6

SUMMARY

The present work deals with “Studies on *Fusarium solani* - *Meloidogyne javanica* disease complex in *Coleus forskohlii* and its management”. Aspect wise summary of the results is presented below:

The main objective of pathogenicity test in the present study was to determine that *M. javanica* / *F. solani* is a pathogen of coleus and also to provide a data on the effective population levels of pathogens which consequently cause plant damage. To determine the inoculum threshold level of root-knot nematode, *M. javanica*, the coleus plants were separately inoculated with different inoculum levels of *M. javanica* (250, 500, 1000, 2000, 4000 and 8000 second stage juveniles (J₂) / kg soil). Results showed that the reduction in plant growth parameters viz., plant length, fresh weight, tuber weight, number of tubers / plant and forskolin content in tubers of coleus (*Coleus forskohlii* var. Selection - K) was directly proportional to the inoculum level of *M. javanica* i.e. with increasing the inoculum level from 250 to 8000 second stage juveniles (J₂) of *M. javanica*, there was a corresponding increase in the reduction of plant growth parameters of coleus except at 250 and 500 J₂, where number of tubers and fresh weight of plants increased, as compared to control. However, there was no significant variation in plant growth parameters at 250 and 500 J₂ / kg soil, although, the significant reduction in plant growth parameters was recorded at and above 1000 J₂ of root-knot nematode / kg soil. It was also observed that with an increase in the level of inoculum there was a progressive increase in host infestation by root-knot nematode as indicated by the number of galls / root system as well as the population of nematode. Moreover, the rate of nematode multiplication was reduced with the increase in the inoculum density of *M. javanica*.

The characteristic symptoms of the root-knot disease appeared on the underground parts of the coleus plant particularly when inoculated at and above 1000 J₂ / kg soil of *M. javanica*. Infected roots developed typical galls of varying
size. In case of multiple infections on the nearby tissues, small galls may oftenly coalesced to form large gall. The galls developed on both lateral as well as tuberous roots of the plants. However, the occurrence of galls was more on the lateral roots as compared to tuberous roots. The galls also appeared in the form of ring like structures on some tuberous roots. Eggs were laid down by the adult females in a gelatinous egg mass which was visible on the infected root surfaces. Sometimes, the presence of spherical white females with egg mass on the surface of fine roots was also noticed. Besides galling in roots, some other typical symptoms in the form of forking of tuberous roots and appearance of galls at the base of the stem near the soil level were also observed at and above 2000 J₂ / kg soil.

The nematode density particularly at 1000 J₂ and above inoculum levels exhibited varying degree of stunted growth in plants accompanied with premature yellowing, drying and shedding of leaves, giving the infected plants an unhealthy appearance. Moreover, at the highest inoculum level, the diseased plants showed temporary day time wilting during hot hours even in the presence of enough soil moisture. The aerial symptoms (yellowing, drying and shedding of leaves) appeared within 8 to 9 weeks at 1000 and 2000 J₂, and 5 to 6 weeks at 4000 and 8000 J₂ after inoculation. However, on the other hand the aerial symptoms did not appear at all at lower inoculum levels of M. javanica (250 and 500 J₂).

From the present findings it could be inferred that the damaging threshold level of M. javanica on C. forskohlii was 1000 J₂ / kg soil as the population at this level was associated with the significant decline in plant growth parameters including yield of tuber and forskolin. This information may provide a base line for further research to develop appropriate and effective management tactics.

The pathogenicity test of F. solani in coleus clearly revealed that there was no significant variation in growth parameters of coleus inoculated with different inoculum levels of F. solani (0.5 to 5.0g mycelium + conidia / kg soil) as compared to uninoculated plants (control). The aerial and root-rot symptoms were also not observed in the coleus plants inoculated with different inoculum levels of
F. solani. It can be concluded from these results that the root-rot fungus, F. solani neither caused any damage nor produced any symptoms in coleus, the reason being that this fungus is not pathogenic to coleus when present alone.

Coleus seedlings were inoculated with M. javanica (1000 J2/kg soil) and F. solani (2.0 g mycelium + conidia/kg soil) either individually or concomitantly as well as sequentially with an interval of 15 days between the nematode or fungal inoculations to determine whether the interaction was concomitant or sequential. The significant reduction in plant growth parameters viz., plant length, fresh weight, tuber weight, number of tubers and forskolin content in tubers of coleus was recorded when M. javanica present alone. However, on the other hand F. solani in coleus did not significantly affect the plant growth parameters. The highest reduction in plant growth parameters and the maximum rotting in coleus were recorded in the plants inoculated simultaneously with M. javanica and F. solani followed by in the plants inoculated sequentially with M. javanica 15 days prior to F. solani and F. solani 15 days prior to M. javanica.

The root-knot nematode multiplication and the development of galls/root system were significantly reduced in presence of F. solani as compared to when the M. javanica was present alone. The greatest reproduction factor and number of galls per root system were recorded in the plants inoculated with M. javanica alone followed by M. javanica 15 days prior to F. solani, F. solani 15 days prior to M. javanica and M. javanica + F. solani. The results also showed F. solani parasitize the females, egg masses and eggs collected from the roots of coleus, whereas, no parasitism was observed in second stage juveniles of M. javanica isolated from the rhizospheric soil of plants inoculated with M. javanica and F. solani either concomitantly or sequentially.

Coleus plants showed both underground (root galling and root-rot) and above ground (collar-rot and crown-rot) symptoms when both M. javanica and F. solani present together, irrespective of whether they were inoculated simultaneously or one after another at 15 days interval. Moreover, the severity of both underground and above ground symptoms were more or less at par in the
plants inoculated either concomitantly (*M. javanica* and *F. solani*) or sequentially (*M. javanica* 15 days prior to *F. solani*). While, the severity of symptoms was quite less in the plants inoculated with one of the sequential inoculations i.e. *F. solani* 15 days prior to *M. javanica*. The symptoms produced by the interaction of *M. javanica* and *F. solani* in various combinations are described as follows:

The leaves present at the base of lower branches of the plant first showed yellowing of leaves which later turned brown to black in colour followed by drying. These symptoms started from the margin and apex of leaves extending inwardly and downwardly, respectively. Finally, it was followed by premature withering and falling of leaves. The yellowing, drying and withering of leaves gradually proceeded upward and then followed by appearance of dark brown to black coloured spot on the stem near the soil line. Later, the spot enlarged and extended upward on stem up to a considerable distance and covering all around the circumference of the infected stem. Further, symptoms appeared in the branches of the affected plants. It first appeared in the lower branches and later to upper branches. Symptoms appeared in the form of brown to black coloured spot, which later extended upward and covered all around the circumference of the infected branch.

Underground symptoms were also observed in the lateral and tuberous roots. Besides, galling on infected roots, it also exhibited the appearance of dark brown to black coloured areas due to rotting leading to varying degree of destruction and deterioration of tissues of lateral and tuberous roots. The infected tubers showing rotting also emitted bad odour. In presence of *F. solani*, the galls formed by the *M. javanica* also showed the symptoms of rotting and deterioration. When the infected branches and tubers were cut split longitudinally, brown to black coloured internal tissues were also observed.

The association of *M. javanica* and *F. solani* not only synergistically reduced the plant growth parameters but also caused the root-rot and induced the expression of collar-rot and crown-rot symptoms. The time involved in expression of these symptoms also varied in different treatments. The symptoms were
recorded earliest in the plants where inoculation was done simultaneously with both the pathogen followed by sequential inoculation of *M. javanica* 15 days prior or later to *F. solani*.

Coleus plants were inoculated with root-knot nematode, *M. javanica* (@1000 J$_2$/kg soil) and varying inoculum levels of root-rot fungus, *F. solani* (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0g mycelium + conidia / kg soil) to find out the minimum inoculum level of *F. solani* required to cause the disease complex and also, its impact on plant growth components (plant length, fresh weight, tuber weight, number of tuber / plant and forskolin content in tuberous roots) as compared to uninoculated plants (control). The inoculation of *M. javanica* @ 1000 J$_2$/kg soil on coleus caused significant reduction in plant growth parameters viz., length, fresh weight, tuber weight, number of tubers and forskolin content. But, no significant variation in plant growth parameters was recorded in coleus inoculated with different inoculum levels of *F. solani* (0.5 - 5.0 g mycelium + conidia / kg soil). The simultaneous inoculation of coleus plants with *M. javanica* and either of the inoculum levels of *F. solani* i.e 0.5, 1.0 and 2.0 g mycelium + conidia / kg soil, exhibited significant greater reduction in plant growth parameters as compared to individual inoculation of *M. javanica*. Further, it was observed that the reduction was increased with an increase in inoculum level of fungus. Coleus plants inoculated with either by *M. javanica* alone or in combination with *F. solani* irrespective of inoculum level of fungus, not only reduced the tuber yield but also reduced the forskolin content in tubers as compared to control. The reduction in tuber weight inturn also caused an overall reduction in yield of forskolin / plant.

The reduction in the reproduction factor and number of galls caused by *M. javanica* was significantly increased with an increase in the inoculum levels of *F. solani* (0.5, 1.0 and 2.0g mycelium +conidia /kg soil). As far as root-rot was concerned it was found to increase with an increase in the inoculum level of *F. solani* when it was present along with *M. javanica*. Similarly, the inoculation of *M. javanica* with either of the inoculum level of *F. solani* (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0g mycelium + conidia / kg soil) showed yellowing, drying and shedding of coleus leaves, which gradually increased with the increase in inoculum level of *F.
In addition to this, association of *M. javanica* with *F. solani* at or above 2.0 g mycelium + conidia/kg soil not only induced the appearance of crown-rot and collar-rot symptoms, but also reduced the number of days required for expression of these symptoms with an increase in the inoculum level of *F. solani*. Further, it was interesting to note that the coleus plants could not survive when inoculated concomitantly with *M. javanica* and either of the higher inoculum levels of *F. solani* (3.0, 4.0 and 5.0 g mycelium + conidia/kg soil).

To the best of my knowledge, the literature available revealed that the association of root-knot nematode (*M. javanica*) and root-rot fungus (*F. solani*) not only constituted the first disease complex of *Coleus forskohlii*, but, also reported as new host for *M. javanica* from India and elsewhere.

The presence of *F. solani* along with *M. javanica*, not only decreased the percentage of penetration and occurrence of different stages in the roots but also subsequently delayed the development of different stages of the nematode in coleus. Fecundity of the females was also found to be reduced with an average of only 168 eggs/egg mass in *M. javanica* and *F. solani* inoculated plants as compared to 315 eggs/egg mass in *M. javanica* alone inoculated plants. The highest number of juveniles were observed in plants inoculated with *M. javanica* alone, followed by in presence of fungus. The percentage of male formation of *M. javanica* was greater in presence of *F. solani* as compared to when *M. javanica* was present alone. The life cycle of *M. javanica* on coleus was completed in 27 days, whereas, the duration of life cycle was adversely affected in the presence of *F. solani* and it took about 37 days to complete the life-cycle. Thus, the presence of *F. solani* delayed the life-cycle of root-knot nematode, *M. javanica* by 10 days.

Since nematodes and fungi are the common inhabitant of soil, their secretions and excretions might have naturally affect each other in various ways. Studies on the effect of culture filtrate of different fungi viz., *T. harzianum*, *T. viride*, *T. aureoviride*, *T. lignorum*, *T. pseudokoningii*, *T. koningii*, *T. hamatum*, *C. pallescens*, *F. solani*, *P. lilacinus* and *P. italicum* on the hatching and mortality of *M. javanica* were therefore carried out. It was observed that the culture filtrates of
these fungi were effective to varying degree in killing the juveniles (J2) and inhibiting the hatching of eggs of *M. javanica*. In general, higher concentrations (S and S/2) of culture filtrate of different fungi showed marked nematicidal effect on *M. javanica*, but this effect was gradually decreased with the increase in the dilution of the culture filtrates. The increase in mortality of juveniles with increase in the exposure time in the culture filtrate has also been observed. The complete suppression of juvenile emergence from the eggs of root-knot nematode was observed in the culture filtrate of *P. lilacinus* at “S” and “S/2” concentrations. In the same concentrations, the maximum inhibition in egg hatching was recorded in the filtrates of the *T. harzianum* followed by *T. viride, T. aureoviride, T. lignorum, T. pseudokoningii, T. koningii* and *T. hamatum*. Similarly, in “S/10” and “S/100” concentration the greatest inhibition in hatching was observed in *T. harzianum* followed by *P. lilacinus, T. viride, T. aureoviride, T. lignorum* and *T. pseudokoningii*. However, on the other hand, it was interesting to note that the lowest concentration (“S/100”) of *T. koningii, T. hamatum, C. pallescens* and *P. italicum* filtrates significantly stimulated the hatching of root-knot nematode.

In general, the culture filtrate of *T. harzianum* showed highest nematicidal effect to *M. javanica* followed by *P. lilacinus, T. viride, T. aureoviride, T. lignorum, T. pseudokoningii, T. koningii, T. hamatum, C. pallescens, F. solani* and *P. italicum* in different concentrations and exposure periods. The culture filtrate of “S” concentration of *P. lilacinus, T. harzianum, T. viride* and *T. aureoviride*, caused cent per cent mortality of root-knot nematode after 96 hours, whereas, in *T. harzianum* cent per cent mortality also occurred after 48 hrs. The cent per cent mortality was also recorded in the “S/2” concentration of culture filtrate of *T. harzianum* and *P. lilacinus* after 96 hours. In the “S/10” concentration after 96 hrs, the significant mortality was recorded only in the culture filtrate of *P. lilacinus, T. viride, T. harzianum, T. harzianum, T. koningii, T. lignorum, T. pseudokoningii* and *T. viride*. However, on the other hand, no mortality was recorded in the filtrates of *C. pallescens, P. italicum T. aureoviride, T. hamatum, T. koningii, T. lignorum* and *T. pseudokoningii* even after 96 hours at “S/100” concentration.
The studies were also conducted on the management of *M. javanica* - *F. solani* disease complex in coleus by using fungal biocontrol agents, organic additives and a fungicide either alone or in different combinations. The results showed that the out of eight fungal biocontrol agents, the individual inoculation of only four biocontrol agents *viz.*, *Trichoderma aureoviride*, *T. harzianum*, *T. lignorum*, and *T. viride* showed significant increase in plant growth parameters (except in number of tubers/plant) as compared to uninoculated plants (control). Similarly, the amendments of organic additives in the form of oil cakes and chopped leaves of plants in soil, only six oil cakes *viz.*, groundnut, karanj, linseed, mohwa, neem and soybean and chopped leaves of three plants *viz.*, french marigold, vasaka and white datura significantly improved the plants growth parameters (except in number of tubers in chopped leaves amended soil) of coleus as compared to plants grown in unamended soil (control).

The coleus plants did not survive when they were inoculated concomitantly with *M. javanica* and *F. solani* or even when the fungal bioagents *viz.*, *T. aureoviride*, *T. hamatum*, *T. koningii*, *T. lignorum* and *T. pseudokoningii* were also inoculated during concomitant inoculation. Moreover, individual inoculation of these fungal bioagents significantly delayed the mortality of plants and appearance of the collar-rot symptoms (except *T. pseudokoningii* treated plant) and crown-rot symptoms as compared to plants inoculated with *M. javanica* and *F. solani*. However, on the other hand, no mortality was observed in coleus plants treated with Bavistin, *P. lilacinus*, *T. harzianum* and *T. viride*. Besides, the respective treatments not only significantly delayed the appearance of collar-rot symptom but also completely suppressed the appearance of crown-rot symptom and showed better plant growth parameters. The significant minimum reduction in plant growth parameters *viz.*, length, fresh weight, tuber weight, number of tubers/plant and forskolin content in tuber was found in the plants treated with *P. lilacinus* followed by Bavistin, *T. viride* and *T. harzianum* as compared to control. The lowest number of galls/root system, reproduction factor of root-knot nematode and percentage root-rot caused by *F. solani* was recorded in the plants treated with *P. lilacinus* followed by Bavistin, *T. viride* and *T. harzianum*. 
The mortality due to infection of *M. javanica* and *F. solani* was also recorded in coleus even when grown in soil amended with chopped leaves of aak, french marigold, lantana, snake weed, white datura, and wild senna. Moreover, mortality of plants did not occur when they were grown in soil amended with oil cakes of groundnut, karanj and neem and chopped leave of vasaka. Besides, the respective treatments also significantly delayed the appearance of collar-rot and crown-rot symptoms in coleus plants. The significant lowest reduction in plant growth parameters *viz.*, fresh weight, tuber weight, number of tubers and forskolin content in tubers was found in plants grown in soil amended with karanj cake followed by neem cake, groundnut cake and chopped leaves of vasaka as compared to unamended and uninoculated plants. However, the minimum reproduction factor and number of galls / root system were recorded in the plants treated with neem cake followed by groundnut, karanj cake and vasaka leaves, whereas, the lowest percentage of root-rot was recorded in the plant grown in soil amended with karanj cake followed by neem cake, groundnut cake and vasaka leaves.

The integration of bare - root dip treatment with Bavistin, inoculation of fungal biocontrol agent(s) *viz.*, *P. lilacinus*, *T. harzianum* and *T. viride* and amendments of either of the oil - cakes (groundnut cake, karanj cake and neem cake) or chopped leaves of vasaka in different combinations in the soil, not only increased the viability of coleus plants but also showed better plant growth parameters by reducing the intensity of disease development in terms of root galling and rotting in coleus as compared to plants untreated and inoculated with *M. javanica* and *F. solani*, where, plants did not survive beyond 81 days. The combination of different treatments used in integrated management also delayed / completely suppressed the appereance of collar and crown-rot symptoms in coleus. The efficacy of Bavistin, fungal bioagnts and organic additives in different combinations for the management of *M. javanica - F. solani* disease complex in coleus was grater in comparisons to when these components were used alone. The integration of various components in different combinations for instance, Bavistin + *P.lilacinus* +*T. viride*, Bavistin + *T. viride* + Neem cake, Bavistin + *P. lilacinus*
+ *T. harzianum* and Bavistin + *P. lilacinus* + neem cake showed better protection against *M. javanica – F. solani* disease complex in coleus. These treatments may be recommended to the farmers only after making field trials for the management of disease complex caused by *M. javanica* and *F. solani* in coleus.