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PLANT PARASITIC NEMATODES AND FUNGI ASSOCIATED WITH WILT DISEASE COMPLEX OF BETELVINE IN NORTHERN KARNATAKA AND ITS MANAGEMENT

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Abstract: *A survey on the occurrence of root-knot disease in three major betelvine growing districts of northern karnataka revealed 0.0 to 87% of disease incidence. The present survey also revealed that the association Meloidogyne spp. with fungi, namely, Sclerotium rolfsii, Rhizoctonia bataticola and Fusarium solani in most of the locations surveyed. Pathogenicity of organisms (M. incognita, S. rolfsii, R. bataticola and F. solani) involved in wilt disease complex of betelvine was proved under glasshouse conditions and all of them were found to be pathogenic. Individually, S. rolfsii was the most aggressive pathogen followed by R. bataticola, F. solani and M. incognita. While combined inocula of all the four organisms (M. incognita + S. rolfsii + R. bataticola + F. solani) was more damaging than the concomitant inoculations of nematode with either of the three fungi. In an integrated management study conducted in a naturally affected betelvine garden, it was found that a combined application of an organic amendment (Enriched farmyard manure) with a biocontrol agent (Trichoderma viride) and chemicals (Carbofuran, Carboxin and Carbendazim) was found to be effective in reducing the wilt incidence, nematode population, number of galls and Competitive Saprophytic Ability (CSA) of fungi namely, S. rolfsii and R. bataticola.*

Key words: *Wilt disease complex, Root-knot disease, Meloidogyne incognita, Sclerotium rolfsii, Fusarium solani, Rhizoctonia bataticola.*

Introduction

Betelvine (*Piper betle* Linn) is one of the important lucrative plantation crops providing higher net returns per unit area and for its wide usage. So far, 39 species within 15 genera of plant parasitic nematodes have been reported to be associated with betelvine crop in India (Bhatt et al., 2004; Jane et al., 2014). The roots of nematode infested vines were found to be affected with other soil-borne plant pathogenic fungi, viz. *Sclerotium rolfsii*, *Phytophthora palmivora*, *Rhizoctonia solani* and *Fusarium solani*. The higher density of nematodes especially, *Meloidogyne incognita* predispose the betel roots for the entry of soil-borne plant pathogens, which may lead to the wilt syndrome (Jonathan et al., 1996). Betelvine acreage in northern Karnataka is steadily coming down due to wilt-like malady (Maiti et al., 1998; Anonymous 2001) and there is no information available on the cause of the disease and its mangement. Some information is available from Tamil Nadu and southern Karnataka on the association of fungal nematode disease complex in betelvine wilt. Considering the heavy economic loss the nematode and fungus cause on betelvine, many attempts have been made for its control (Anonymous, 1984). These include cultural, physical, chemical, biological, host resistance, use of organic amendments and integrated methods. Of all the available methods, the most extensively tried and effective method is chemical control. Although chemicals are effective, plant protection in the betelvine gardens, as a whole is difficult proposition because of the fact that the produce is a green leaf consumed when fresh, and harvested at very short intervals (a week to 15 days) which does not permit use of toxic chemicals. The situation emphasizes the reduced use of chemicals for checking disease of betelvine. So, there is need to develop an eco friendly, economical and alternative method for effective management of wilt-disease complex of betelvine. Hence, the present investigations were taken up to identify the organisms associated with

the wilt disease complex of betelvine in northern Karnataka and also to study the feasibility of using biocontrol agent, organic amendments and chemicals in the management of wilt disease complex of betelvine.

Materials and Methods

A survey was undertaken in the major betelvine growing districts (Haveri, Koppal and Bagalkot) of northern Karnataka from 2003-2004. Soil and roots samples were collected from sufficiently wet fields from the rhizosphere of betelvine crop. In the similar manner, a total of about 10 to 15 spots were selected randomly for taking soil and root samples representing the whole field. Later from this, composite samples of 200 g of soil and 5 g of root were formed. Randomly, 100 plants were selected in different locations in a field and number of plants wilted was counted and the mean wilt incidence was expressed in percentage. The wilt symptoms were identified based on key provided by Maiti and Sen (1979). Galled root system was scored by using a disease rating scale (0 to 5 scale) given by Taylor and Sasser (1978). The disease severity (root knot disease) was calculated for the village by using following formula.

$$\text{Disease severity} = \frac{\text{Number of infected root samples from a village}}{\text{Total number of root samples collected from a village}} \text{X100}$$

Cobb's sieving and decanting technique was followed for the estimation of nematode population in the soil. The population densities of different nematode species in the samples were calculated using the formulae given by Norton, 1978.

Identification of pathogens associated with the wilt complex:

S. rolfsii Sacc. fungus was identified based on the morphological and cultural characters described by Domsch et al. (1980). The morphological, cultural and formation of sclerotia were the principal characters to identify the pure cultures of *R. bataticola*. The characters were compared with those described by Ashby (1927) and the fungus was identified as *R. bataticola*. The morphology of the another fungus isolated from diseased stem of betelvine was studied with respect to its shape and size of macro-conidia, micro-conidia, chlamydospores; and colour of the colony. The fungus was identified as *Fusarium Solani* based on the descriptions given by Siddappa (1984). Sand-corn meal medium was prepared for the multiplication of pure cultures of *S. rolfsii, R. bataticola* and *F. solani* were maintained for proving the pathogenicty of each fungi.

Proving the pathogenicity of wilt - inducing fungi and root knot nematode

The betelvine variety 'Ambadi' raised in earthen pots containing sterilized soil to which different inocula (S. *rolfsii, R. bataticola* and *F. solaru)* maintained on sand-corn meal medium, were added @ 4 per cent in following combinations (S. *rolfsii* alone, *R. bataticola* alone, *F. solani* alone, Control (No inoculum was added). The pots were maintained at 25 per cent moisture holding capacity and the moisture loss was maintained by adding water on weight basis. Observations were made every alternate day regarding development of wilt symptoms. After the plants showed wilt symptoms such plants were carefully uprooted and the fungi were reisolated by standard tissue isolation method. The fungi reisolated were compared with original culture. Root-knot infected tomato plants were collected from the orchards of College of Agriculture, Dharwad. Root portion was carefully removed from the soil and washed gently under running tap water. Egg masses were picked and kept for hatching in water in a Petri dish. After 24-36 hours, juveniles hatched and the same were used to inoculate tomato, bitter gourd and betelvine grown in sterilized soil-sand mixture in greenhouse. These plants served as culture plants.

The plants were depotted carefully. The root systems were washed free of soil, the galls containing eggmasses were used to get inoculum of the pathogen for further studies throughout. The identification of the root knot nematode species was made on the basis of characters of perineal pattern described by Eisenback *et al.* (1981). Thirty days after planting betelvine cuttings in test pots, the suspension containing pre-determined number of juveniles was pipetted and spread uniformly over the surface of roots which were carefully exposed earlier. Then the roots were covered with soil. The plants were lightly watered to keep the soil moist. A similar treatment was given to the uninoculated check plants except that only water was used instead of the nematode suspension. After giving sufficient time (60 to 100 days) to complete 2 to 3 generations of the nematode, the root systems of the depotted plants were washed free of soil and was examined for presence of galls. For further confirmation, staining of root material was done with acid fuchsin (McBeth *et al,* 1941).

Interaction studies

To study the effect of simultaneous inoculation of *M. incognita, S. rolfsii, R. bataticola* and *F. solani* either singly or in various combinations on plant growth, host infestation, nematode multiplication and disease development, a pot culture experiment was designed under greenhouse conditions. Fifty grams of each fungal giant culture or one thousand freshly hatched second stage juveniles of root knot nematode was applied individually to the 150 day old betelvine plants cv. Ambadi grown in 1:1 sterile sand soil mixture in 45 cm diameter earthen pots. Inoculum of all the said pathogens was applied singly or in combinations as per the following treatments (Control, M. *incognita* alone, S. *rolfsii* alone, *M. incognita* + *S. rolfsii,* R. *bataticola* alone, *M. incognita* + R. *bataticola, F. solani* alone, *M. incognita* + *F. solani, M. incognita* + *S. rolfsii* + R. *bataticola* + *F. solani).* Inoculations using both the pathogens (nematode and fungi) were done as per the method described by Sitaramaiah and Parvathi Devi (1990). Observations on wilting and other symptoms were recorded till 150 days after inoculation with the said organisms. Data on shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, number of galls/plant, final population of nematodes in soil and per cent wilt incidence were recorded, whereas, rating for gall index was done following the scale suggested by Tayler and Sasser (1978) as given below:

No galls or eggmass	0
1 to 2 galls or eggmasses	1
3 to 10 galls or eggmasses	2
11 to 30 galls or eggmasses	3
31 to 100 galls or eggmasses	4
More than 100 galls or eggmasses	5

The data obtained in the present investigation for various parameters such as shoot and root lengths, fresh and dry shoot weights, fresh and dry root weights, root gall index and final nematode population were subjected to ANOVA for a completely randomized design for *invitro* pot culture studies.

Integrated management of wilt disease complex of betelvine by integrating organic amendments (neem cake, farmyard manure), biocontrol agent *(trichoderma viride)* and chemicals (carbofuran, carboxin and carbendazim)

A field trial was carried out (during June 2003-Febrauary2004) in natural wilt disease complex affected betelvine garden (4 year old) in Banuvalli village of Harihar taluk (Karnataka). An affected area of 300 square meters was selected in the garden, as evidenced by mortality of vines here and there in the area. This site was further divided in to twenty equal sized plots of 10 x 1.5 m. Each plot had 24 vines (including dead/wilted vines). The number of dead/wilted vines was recorded. The following treatments were imposed in randomized block design. Each treatment was replicated four times. Observations on per cent wilt incidence, nematode population, number of galls /5 g of root and Competitive Saprophytic Ability (%) of *R. bataticola* and S. *rolfsii* were recorded at 90 days after imposing the treatments. The data obtained in the present

investigation for various parameters such as shoot and root lengths, fresh and dry shoot weights, fresh and dry root weights, root gall index and final nematode population were subjected to ANOVA for a randomized block design for *in vivo* field studies.

Results and Discussion

An extensive survey was taken up in 33 gardens belonging to 14 villages affected by wilt disease in the three major betelvine growing districts of northern Karnataka. Wilting due to foot rot/collar rot causing fungi, viz. *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Fusarium solani* was noticed in many locations. Freshly wilted vines showed root galling due to root knot nematode, *Meloidogyne incognita* in many locations. On the whole, *Rhizoctonia* (collar) rot was widely prevalent in the areas surveyed (18%) with the incidences of *S. rolfsii* and *F. solani* being minimum (0.7% and 0.4% respectively). Root knot disease showed an incidence of 18%. Maximum malady was seen in Haveri district followed by Koppal and Bagalkot districts (data not shown). However, earlier surveys in Karnataka revealed the presence of *Meloidogyne* with an average disease incidence of 30% in some areas (Anonymous, 1984). The prevailing root-knot nematode species in northern Karnataka was characterized by the presence of high, squarish dorsal arch that often contained a distinct whorl in the tail terminal area. The striae were smooth to wavy, sometimes zig-zagged and distinct lateral lines were absent. The present survey revealed that *Meloidogyne incognita*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Fusarium solani* to be most commonly associated with betelvine crop. These results are in confirmation with the findings of the survey (1983-84) conducted by AICRP (Betelvine) in Karnataka. The present survey also indicated association of *Meloidogyne* with fungi namely, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Fusarium* in most of the locations surveyed, with a high frequency of occurrence of both the pathogens (*Meloidogyne incognita* with any of the three fungi) from soil and root samples collected from Haveri district. Similar observations were also made in survey conducted by AICRP (Betelvine) in Orissa (Anonymous, 1995). Soil and root samples collected during the survey from different parts of the northern Karnataka revealed the presence of plant parasitic nematodes like *Aphelenchus* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne* spp., *Radopholus* spp., *Rotylenchulus reniformis*, *Pratylenchus* spp. and *Xiphinema* spp (Table 1). The root knot nematode was found to be most predominant species in the soil sample collected from different cultivars. This was followed by reniform nematode (*Rotylenchulus reniformis*), root lesion nematode (*Pratylenchus* spp) and *Helicotylenchus* spp. The higher density of nematodes especially *Meloidogyne incognita* and *Rotylenchulus reniformis* predispose the betel roots for the entry of soil borne plant pathogens, which may lead to the aggravated wilt syndrome. The results are in confirmation with the results obtained by Sivakumar and Marimuthu (1984) who have reported similar nematode genera in their survey of nematode parasites associated with betelvine in Tamil Nadu.

In the pot culture experiment, wilt symptoms were first recorded at 30 days after inoculation in treatment receiving a combined inocula of all the four organisms *(M. incognita + S. rolfsii + R. bataticola + F. solani).* Whereas in case of combined inoculations involving nematode and a fungus *(S. rolfsii* or *R. bataticola* or *F. solani)* the wilt symptoms were first recorded in concomitant inoculation involving *M. incognita* + S. *rolfsii* (55 days after inoculation) followed by *M. incognita* + R. *bataticola* (90 days after inoculation) and *M. incognita* + *F. solani* (110 days after inoculation) (Table 2). However, in individual inoculations of fungi, the wilt symptoms were first recorded in S. *rolfsii* (65 days after inoculation), followed by *R. bataticola* (110 days after inoculation) and *F. solani* (150 days after inoculation). Significant reduction in shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight was noticed in all the treatments in comparison to uninoculated control. Data presented in table 3 revealed that the four organisms *(M. incognita, S. rolfsii, R. bataticola* and *F. solani)* adversely affected plant growth parameters

like shoot and root length, fresh and dry weight of shoot, fresh and dry weight of root per plant. Of the four organisms inoculated individually, S. *rolfsii* caused greater reduction in plant growth parameters over control than caused either by *R. bataticola,* or *F. solani* or *M. incognita.* But reduction in plant growth parameters caused by fungi viz. *R. bataticola, F. solani* and nematode (*M. incognita*) were on par with each other (Table 3). In cases of combined inoculations with nematode and fungi, greatest reduction in growth parameters was caused by the *M. incognita* + *S. rolfsii* followed by the *M. incognita* + *R. bataticola* and *M. incognita* + *F. solani.* However, the treatment receiving the inoculum of all the four organisms, showed a highest reduction in plant growth parameters as compared to other treatments (Table 3). In these interactions, it was observed that the effect of the combined inocula *(M. incognita* and either of the three fungi) on plant growth parameters was additive in nature, where inoculations were simultaneous wherein the resultant effect on growth parameters was almost equal to sum total of individual effects. However in treatment receiving a combined inocula of all the four organisms *(M. incognita* + *S. rolfsii* + *R. bataticola* + *F. solani*), the resultant effect was more than the simple additive effect. Root knot index (5.00) as well as juvenile population in soil (1011.28) was more in nematode-alone treatment. Whereas, in case of concomitant inoculations involving nematode and fungi, the lowest root knot index and nematode population were recorded in the association treatment of *M. incognita* + *S. rolfsii* followed by *M. incognita* + *R. bataticola* and *M. incognita* + *F. solani.* In general, the treatment receiving a combined inoculation of all the four organisms recorded significantly lowest root knot index and nematode population over other treatments.

The results of integrated management of wilt disease complex betelvine clearly indicated that the wilt incidence, nematode population, number of galls and Competitive Saprophytic Ability per cent of *R. bataticola* and S. *rolfsii* were minimum is the plots where enriched farmyard manure (15 kg/15 m2), *T. viride* (200 g/15 m2), carbofuran 3G (500 kg/15 m2), carboxin 75 WP and carbendazim 50 WP (0.1%) were integrated followed by the combined application of neem cake (1.5 kg/ 15 m2) and above mentioned chemicals (Table 4). Similar results were obtained in a field experiment conducted by AICRP (Betelvine) in Tamil Nadu, where the wilt per cent and root knot index were minimum in a treatment involving a combination of carbofuran @ 1.5 kg a.i./ha; neem cake 50 N/ha and 0.5% Bordeaux mixture (Anon., 1984). Krishna Rao (1994) also reported the efficacy of integration of physical, chemical and biological methods on nematode fungal complex of chickpea. Field evaluation of fungicides by several workers have shown that carboxin or carbendazim as soil drench was effective against, S. *rolfsii* and *R. bataticola* on many crops (Mishra and Ghosh, 1978; Tarabeih and Attarackehi, 1979).However, all the treatments comprising chemicals significantly controlled the disease complex compared to other treatment combinations whereas soil application of organic amendments (enriched FYM + neem cake) showed no significant effect in reducing the either wilt incidence or nematode population or number of galls or CSA per cent of the fungi namely S. *rolfsii* and *R. bataticola.* The studies conducted by Nishatkhalis and Manoharachary (1985) revealed that neem cake was found to be ineffective in earlier stages which may be due to the slow degradation of oilcake. Raju (1997) also reported that the application of neem cake (250 g rrr2) was not effective in reducing the wilt incidence of crossandra. In the present study, it was observed that, the neem cakes applied to the plots were not fully degraded. This may be the reason for the ineffectivity of organic amendments in the control of wilt disease complex of betelvine.

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Table 1: Community analysis of plant parasitic nematodes associated with betelvine

Nematode species	Absolute frequency	Absolute density	Relative frequency	Relative density	Prominence value
Aphelenchus spp.	13.00	4.29	19.25	2.08	0.20
Helicotylenchus spp.	47.82	28.62	12.37	11.80	1.97
Hoplolaimus spp.	8.69	1.34	0.67	0.32	0.04
Longidorus spp.	13.04	6.48	1.84	2.70	0.23
Meloidogyne spp.	78.26	85.57	28.77	25.56	7.56
Pratylenchus spp.	56.52	33.84	13.55	13.21	2.63
Radopholus spp.	21.73	2.99	1.28	1.34	1.40
Rotylenchulus reniformis	65.21	38.11	17.79	17.67	3.18
Scutellonema spp.	12.00	1.90	0.90	0.49	0.06
Tylenchus-like PPN	43.47	15.23	4.94	8.31	1.00
Xiphinema spp.	34.78	10.27	4.24	5.47	0.60
Other Dorylaimid PPN	86.95	27.40	7.38	11.82	2.55

Table 2: Influence of single or combined inoculations with *M. incognita, S. rolfsii, R. bataticola* and *F. solani* on per cent wilt incidence in betelvine (Average of 4 replications)

Treatment	Days after inoculation								
	30	45	55	65	75	90	110	150	160
C	--	--	--	--	--	--	--	--	--
N	--	--	--	--	--	--	--	--	--
S	--	--	--	25	50	75	100	--	--
NS	--	--	25	75	100	--	--	--	--
R	--	--	--	--	--	--	25	75	100
NR	--	--	--	--	--	50	75	100	--
F	--	--	--	--	--	--	--	25	25
NF	--	--	--	--	--	--	25	50	50
NSRF	25	50	75	100	--	--	--	--	--

C = Uninoculated control
N = *M. incognita*
S = S. rolfsii alone
NS = *M. incognita* + *S. rolfsii*
R = *R. bataticola* alone
NR = *M. incognita* + *R. bataticola*
F = *F. solani* alone
NF = *M. incognita* + *F. solani*
NSRF = *M. incognita* + *S. rolfsii* + *R. bataticola* + *F. solani*

Table 3: Influence of single or combined inoculations with *M. incognita*, *S. rolfsii*, R. *bataticola* and *F. solani* on plant growth parameters, root knot index and nematode population in betelvine (cv. Ambadi) (Average of 4 replications)

Treatment	Length (cm)		Shoot weight (g)		Root weight (g)		Root-knot index	Final nematode population/ 200 cc of soil
	Shoot	Root	Fresh	Dry	Fresh	Dry		
C	198.41	27.43	64.06	27.13	28.23	3.33	-	-
N	193.77	25.70	56.05	22.66	22.43	2.83	5.00	1011.28
S	185.41	23.23	53.05	20.31	19.65	2.11	-	-
NS	175.90	20.14	47.29	16.91	16.09	1.05	3.13	638.74
R	191.12	25.81	55.10	22.02	21.50	2.22	-	-
NR	181.52	21.63	50.61	18.73	18.01	1.58	3.70	844.48
F	190.84	25.19	55.10	21.96	21.66	2.28	-	-
NF	180.51	21.68	49.96	18.75	18.23	1.33	3.74	821.54
NSRF	170.80	18.26	36.71	14.09	13.43	0.95	2.60	409.45
SEm ±	0.96	0.25	0.46	0.24	0.30	0.01	0.02	7.83
CD at 1%	3.79	0.99	1.83	0.96	1.20	0.04	0.08	30.96

Table 4: Integrated management of betelvine wilt disease complex: Influence of organic amendments (Neemcake, Farmyard manure), biocontrol agent (*Trichoderma viride*) and chemicals (Carbofuran, Carboxin and Carbendazim)*

Treatments	Per cent wilt incidence	Population of root knot juveniles/ 200 cc of soil	No. of galls/ 5 g of root	CSA (%)**	
				R. bataticola	*S. rolfsii*
T_1- Enriched farmyard manure @ 10 t/ha + *T. viride* @ 130 kg/ ha soil application.	21.09	946.30	24.80	37.25	15.50
T_2-Enriched farmyard manure @ 10 t/ha + Neem cake @ 1 t/ha soil application.	29.36	13337.40	35.38	47.25	17.75
T_3-T1 + Carbofuran @ 1 kg ai/ ha + Carboxin (0.1%)	11.43	484.18	16.68	21.38	8.25
T_4-T2+ Carbofuran @ 1 kg ai/ha)+ Carboxin (0.1%) + Carbendazim (0.1%) Soil application	24.48	561.15	22.38	28.38	10.75
T_5-Control	46.43	1447.85	38.13	52.31	19.75
SEm±	5.85	36.23	1.55	1.84	0.61
CD at 5%	18.04	111.67	4.77	5.67	1.87

*Observations recorded at 240 days after imposition of treatments.

** Competitive Saprophytic Ability