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Studies on fungi associated with the root (wilt) disease of the coconut palm

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Root (wilt) disease, one of the major diseases of coconut in India was first investigated by Butler (1908). He isolated a species of *Botryodiplodia* from the rotten roots of the infected palms and considered that the root rot caused by the fungus was probably sufficient to produce the disease. The investigations carried on by Menon and Nair (1951) revealed the complex nature of the disease which in many respects was comparable to the 'Unknown disease' or "Lethal yellowing" in Jamaica and 'Cadang-cadang' or "Yellow Mottle Decline" disease of the Philippines. They, however, observed the constant occurrence of *Rhizoctonia bataticola*, *Rhizoctonia solani* and *Botryodiplodia theobromae* in diseased roots.

The root (wilt) disease of coconuts in Kerala is characterised by the general wilting of the leaves with flaccidity, ribbing and necrosis of the leaves. Shedding of immature nuts is an initial symptom in a majority of the bearing trees. The diseased tree produces small spathes with fewer female flowers and consequently smaller number of nuts. Most of the rootlets and the main roots start drying from their tip backwards. Cracks, blotches, etc., are visible on older regions of the root; the cortex turns brownish in colour and dries up in flakes. In certain instances where the roots remain apparently healthy, even in the advanced stages of disease their absorbing region gets covered with a hard hypodermis that results in cessation of normal functions. Production of new roots decreases with the progress of the disease.

The present investigations are mostly confined to the importance of occurrence of different root-infecting fungi associated with the root (wilt) disease.

MATERIALS AND METHODS

The general laboratory technique followed was that of Riker and Riker (1936) for the preparation of media, maintenance of cultures etc. Sampling of roots for isolation work was made from palms standing in the Research Station farm i.e., from a diseased tract. The root bits were surface sterilized with 1/14 calcium hypochlorite and were plated on different agar media. The fungi growing out from the root material were subcultured on potato-dextrose agar. Roots of other crops such as cowpea (*Vigna sinensis*), Sunnhemp (*Crotalaria juncea*), tur (*Cajanus indicus*) and groundnut (*Arachis hypogaea*) were collected after growing them around the bases of healthy and diseased palms. The root system of the above crops also was examined at fixed intervals and isolations from root bits were carried out.

EXPERIMENTAL

Occurrence and isolation of the pathogen :

a) The roots from healthy and diseased palms were collected and brought to the laboratory for thorough examination and isolation of fungi colonizing the roots. Under the microscope the cortical regions of the affected* root were found to be interspersed with fungal hyphae. In a few roots the epidermal peelings showed the existence of numerous black sclerotia of *Rhizoctonia bataticola*, and microscopic examination of the material showed that the fungal ramifications had grown inside the tissues as hyphae and sclerotia. The healthy roots were devoid of any discoloration and free from fungal growth. Bits from both healthy and affected roots were plated for culturing the fungi present in them.

The results revealed that only affected roots yielded *Rhizoctonia solani*, about 31 per cent of the root bits yielding this fungus. About 5 per cent of the root pieces gave *R. bataticola* and 13.9 per cent of the root bits yielded *B. theobromae*. The percentage occurrence of *R. bataticola* and *trichoderma* sp. were 10.3 and 8.7 respectively in the roots of healthy palms (Table I).

b) To have a clear appraisal of the fungal flora in the root system of a single palm, it was proposed to isolate fungi colonising both healthy and affected roots of a diseased palm.

The palm which was selected for the above study was about 25 years old and possessed all the characteristic symptoms of root (wilt) disease. The outer leaves were yellow and the discoloration was developed at the tip of the leaf and extended backwards. General wilting of the leaves, flaccidity and ribbing of leaflets and necrosis of the leaf tips were also prevalent in all leaves. 1/8th sector of the root system of the palm was exposed and the soil was removed with a powerful jet of water, the soil being washed down into

* Roots showing symptoms of disease

trenches. The diseased condition of the root system was indicated more in the horizontal roots and the vertical roots seemed to be apparently healthy. A total of about 55 affected roots and 12 healthy roots were exposed and isolation of fungi from these roots were carried out as in (a).

The percentage occurrence of *R. solani* was 27.39 in affected roots whereas it did not occur in healthy roots. The occurrence of *B. theobromae* in affected roots was found to be 30.65 per cent while in healthy, the percentage was only 15.22. Greater percentage of *Penicillia*, viz., 13.52 was found to occur in healthy roots (Table II).

c) In the light of the above results it was decided to conduct a comparative study on the percentage occurrence of different genera of fungi in healthy and diseased palms and also to see whether any correlation exists between the diseased condition of the roots and the occurrence of *Rhizoctonia solani*. As far as possible diseased seedlings of the identical ages were compared. The seedlings possessed all the characteristic symptoms of wilt disease.

It was observed that root decay is a common phenomenon in both healthy and diseased seedlings but the percentage of root decay in diseased seedlings was found to be higher, namely 85 in the diseased and 30 in the healthy. The percentage occurrence of *R. solani* ranged from 29.46 to 81.78 in diseased and from the affected roots of healthy seedlings it ranged from 0 to 7.24. On comparing the fungi isolated from healthy and diseased seedlings, it was found that *R. solani* occurs only in affected roots of both healthy and diseased seedlings and *Trichoderma* sp. was the predominant fungus in healthy seedlings (Table III).

ISOLATION FROM INTERCROPS

d) The ability of the fungus to colonize the roots of crops other than the coconut was also studied. Groundnut (*Arachis hypogaea*), cowpea (*Vigna sinensis*) and tur (*Cajanus indicus*) were grown as intercrops in Blocks V & VII of the Research Station compound. The seeds were also sown around the base of healthy and diseased palms, the roots of the seedlings were periodically examined and the fungi colonizing these were isolated.

Isolations from the root bits of one month old seedlings showed the presence of *R. bataticola*, *B. theobromae*, *Aspergillus* spp., *Penicillium* spp. etc. although none of them developed any wilt symptoms viz., yellowing and drooping of the leaves. When the seedlings were two months old they developed slight yellowing and drooping and the roots were found to harbour greater number of *R. bataticola* (Table IV). A few of the root bits were found to yield a sterile fungus with brown mycelia which was identified as a strain of *Corticium solani* by the Commonwealth Mycological Institute, Kew, Surrey,

London. The other fungi which were of frequent occurrence were *Neocosmopora vasinfecta*, *Fusarium* spp., *Pestalotia* spp., *Aspergillus* spp., and *Penicillium* spp.

DISCUSSION

According to the present investigation, preliminary isolations from healthy and affected roots of healthy and diseased coconut palms reveal the existence of different genera of fungi in varying percentages. It is interesting to note that *R. solani* is always associated with the affected roots, about 31 per cent of root bits yielding this fungus. The others, viz., *R. bataticola*, *B. theobromae*, *Pestalotia*, *N. vasinfecta*, *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Chaetomium* and *Trichoderma* sp. are cosmopolitan in their occurrence in healthy as well as in affected roots. The percentage occurrence of *B. theobromae*, however, is greater in affected roots.

The comprehensive study on fungi occurring in healthy and affected roots of a wilted palm also gives evidence of a specific relationship of *R. solani* with the disease incidence in the root. The percentage occurrence of this fungus was observed to be 27.39. The occurrence of *B. theobromae* is remarkably increased in affected roots and the percentage is recorded as 30.65. In healthy root the percentage of this fungus is reduced to 15.22. No perceptible variation in the occurrence of *R. bataticola* was noticed; it ranged from 3.07 and 4.38 in affected and healthy roots respectively. The other fungi are frequent inhabitants of both healthy and affected roots.

The comparative study on the root system of healthy as well as diseased [root (wilt) diseased] seedlings reveals that the occurrence of *R. solani* is met with only in affected roots of both categories of palms and its percentage occurrence varies from 29.46 to 81.74 in diseased, and from 0 to 7.24 in healthy seedlings (Table III). Radha and Menon (1954) reported the occurrence of *R. solani* in the rhizosphere soils of diseased tracts only.

The results on the isolations of fungi from roots of intercrops also reveal that almost all fungi are common inhabitants in the roots of coconut and its common intercrops. *R. bataticola* is found to exist in both healthy and affected roots. The existence of *R. bataticola* in healthy and in affected roots indicates the endophytic nature of its growth which may pass on to the parasitic phase under unfavourable environmental conditions. This phenomenon was also observed by Steinmann (1928) and Hopkins (1929) who found the mycorrhizal relationship of *R. bataticola* and *Sclerotium bataticola* with plant roots like tea and citrus, causing root diseases under conditions unfavourable to the host. Samual and Greaney (1937) refer to such fungi as 'weak parasite'.

SUMMARY

The root (wilt) disease of coconuts is wide spread and severe in occurrence in South India,

R. Solani was found to be associated with roots showing symptoms of disease while other fungi like *R. bataticola*, *B. theobromae*, *Pestalotia* sp., *N. vasinfecta*, *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Chaetomium* sp. and *Trichoderma* sp. were of cosmopolitan occurrence in healthy as well as affected roots.

All the above mentioned fungi are common inhabitants in the roots of the common intercrops in coconut gardens.

R. bataticola was found to exist in the root system of both healthy and diseased palms, whereas occurrence of *R. solani* was restricted in the affected roots.

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TABLE I

Showing fungi occurring in affected and healthy roots
(random sampling) of coconut

Percentage of root bits yielding the various fungi										
Nature of root	<i>R. solani</i>	<i>R. bataticola</i>	<i>B. theobromae</i>	<i>Pestalotia</i>	<i>N. Vasinfecta</i>	<i>Fusarium</i> spp.	<i>Penicillium</i> spp.	<i>Aspergillus</i> spp.	<i>Chaetomium</i> spp.	<i>Trichoderma</i> sp.
Affected root	31.0	5.0	13.0	17.9	15.4	3.5	4.5	2.4	3.5	2.9
Healthy root	0.0	10.3	11.1	33.7	16.9	2.7	13.0	1.1	2.5	8.7

TABLE II

Showing the percentage occurrence of fungi in the roots of wilted palm

Number of root bits	Affected roots	Healthy roots
	55	12
	1595	525
<i>Organisms:-</i>		
<i>R. solani</i>	27.39	0.0
<i>R. bataticola</i>	3.07	4.38
<i>B. theobromae</i>	30.65	15.22
<i>Fusarium</i> spp.	3.00	6.66
<i>Pestalotia</i>	12.03	8.57
<i>N. vasinfecta</i>	0.37	0.19
<i>Aspergillus</i> spp.	3.63	5.90
<i>Penicillium</i> spp.	8.90	13.52
<i>Chaetomium</i> spp.	0.68	0.40
<i>Trichoderma</i> sp.	9.71	10.85
<i>Rhizopus</i>	2.82	4.95
<i>Sphaeriales</i>	0.31	0.91
<i>Gloeosporium</i> sp.	1.25	0.38
<i>Curvularia</i>	0.37	0.0
<i>G. roseum</i>	0.37	0.0
<i>Thielaviopsis</i> spp.	4.20	0.0
<i>Helminthosporium</i> spp.	0.13	0.0

TABLE III

Showing the percentage occurrence of fungi in healthy and diseased seedlings

Condition of seedlings	No. of roots	Nature of roots	Number of root bits	<i>R. solani</i>	<i>R. bataticola</i>	<i>B. theobromae</i>	<i>Pestalotia</i> sp.	<i>Chaetomium</i> sp.	<i>Fusarium</i> spp.	<i>N. vasinflecta</i>	<i>Helminthosporium</i> spp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Trichoderma</i> sp.
Healthy	183	147AR	1440	7.24	3.00	17.64	38.54	11.60	77.68	Nil	Nil	3.60	9.20	22.32
"	60	36HR	260	Nil	Nil	Nil	16.80	Nil	66.66	Nil	Nil	1.10	15.70	80.16
"	91	3AR	30	Nil	15.09	25.09	Nil	Nil	Nil	Nil	Nil	4.30	8.40	33.30
"		57HR	602	Nil	1.76	Nil	Nil	Nil	23.80	Nil	Nil	5.60	18.10	77.68
"		6AR	60	Nil	2.80	15.67	Nil	25.00	50.00	Nil	Nil	2.70	7.80	15.26
"		85HR	850	Nil	Nil	Nil	Nil	1.20	3.75	1.10	1.00	6.20	16.40	59.50
Diseased	174	155AR	1165	29.46	5.64	50.70	41.28	34.02	5.55	Nil	3.77	3.60	8.90	2.24
"	73	19HR	185	Nil	1.89	10.40	28.57	Nil	Nil	Nil	Nil	5.90	13.52	83.74
"		45AR	452	42.15	3.58	50.00	Nil	30.99	48.84	22.19	Nil	2.40	4.50	7.82
"		28HR	278	Nil	Nil	16.35	Nil	5.60	46.26	28.28	Nil	6.40	13.00	47.62
"	42	42AR	310	81.74	20.74	11.90	Nil	31.39	22.00	Nil	Nil	1.79	10.48	19.78
"		OHR	0	0	0	0	0	0	0	0	0	0	0	0

AR = affected roots

• HR = healthy roots

TABLE IV

Showing the percentage occurrence of fungi in intercrops

Intercrops	<i>R. bataticola</i>	<i>R. solani</i>	<i>D. theobromae</i>	<i>N. vasinfecta</i>	<i>Fusarium</i> spp.	<i>Pestalotia</i> sp.	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	<i>Trichoderma</i> sp.
Groundnut	28.33	0.03	5.18	1.50	4.50	0.66	32.16	37.83	5.66
Cowpea	39.16	1.81	2.68	10.50	14.73	1.33	3.33	1.35	1.83
Tur	38.78	0.33	4.33	13.00	14.83	2.66	9.33	12.16	5.66