

ween surface temperature and water vapour concentration, and day-to-day variation of water vapour is highly related to local metrological conditions. At higher surface temperature, water vapour concentration is higher. Although few records are available at Maitri, the trend observed is an important indicator of climate change. These changes in a small region in Antarctica demonstrate that the future of regional climate change may probably be one dominated by regional stimulation and feedback processes. The same may be true for more populated and polluted areas of the globe.

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## Variations in hosting beneficial plant-associated microorganisms by root (wilt)-diseased and field-tolerant coconut palms of West Coast Tall variety

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**Coconut palms (*Cocos nucifera*) in Kerala, India are affected by root (wilt), a debilitating disease caused by phytoplasma, resulting in substantial yield loss. One of the strategies to combat this problem involves breeding for root (wilt)-resistant/tolerant palms by exploiting the genetics of disease escapees identified in the heavily diseased tracts. A preliminary study on the rhizosphere microflora of these disease escapees and diseased coconut palms revealed that the bacterial population ranged from 6.2 to 12.4 × 10<sup>5</sup> cfu/g dry soil in rhizosphere**

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of root (wilt)-diseased palms, while it was only  $1.0$  to  $8.3 \times 10^5$  in field-tolerant palms. The population of actinomycetes was higher in field-tolerant palms, and fungi were on par. In function-specific microbes, the number of cellulose degraders and phosphate solubilizers did not differ much in both diseased and field-tolerant palms. However, significantly higher counts of  $N_2$ -fixers (maximum of  $104 \times 10^2$  cfu/g dry soil) and silicate solubilizers ( $6 \times 10^2$  cfu/g dry soil) were recorded in field-tolerant palms compared to diseased palms (maximum of  $34 \times 10^2$  and  $1.0 \times 10^2$  cfu/g dry soil of  $N_2$ -fixers and silicate solubilizers respectively). At 25–50 cm depth, overall microbial counts in both the diseased and field-tolerant root (wilt) palms were lesser than at 0–25 cm depth. Though the diseased palms had higher bacterial population, they included only 0.21% of beneficial microbes, while the root (wilt)-tolerant palms had overall less bacterial numbers but included 3.6% of beneficial microorganisms. The occurrence of higher population of beneficial plant-associated microbes, particularly silicate solubilizers, in the rhizosphere of disease-tolerant palms and the probable mechanisms they endow to such palms to evade the phytoplasmal infection are discussed here.

**Keywords:** Coconut palms, field-tolerant palms,  $N_2$ -fixers, root wilt, silicate solubilizers.

COCONUT palms in the southern districts of Kerala, India are affected by root (wilt), a debilitating disease caused by phytoplasma (earlier known as mycoplasma-like organisms)<sup>1</sup>. The loss due to this disease has been estimated to the tune of 968 million nuts per year<sup>2</sup>. A leafhopper, *Proutista moesta*, and a lacewing bug, *Stephanitis typica*, were identified as the vectors of the phytoplasma<sup>3,4</sup>. The root (wilt) affected palms are also prone to leaf rot disease, which is caused by a fungal complex<sup>5</sup>. One of the strategies to combat this problem involves breeding for root (wilt)-resistant palms by exploiting the genetics of disease-free, high-yielding palms identified in the heavily infected tracts<sup>6,7</sup>, described here as field-tolerant palms. An attempt was made to unravel the rhizosphere microbial ecology of the root (wilt)-diseased and field-tolerant coconut palms (Figure 1) and the salient findings are presented here.

Root (wilt) disease-free and high-yielding coconut palms (West Coast Tall (WCT) var.) were identified in the hotspot areas of Kottayam, Pathanamthitta and Alappuzha districts of Kerala<sup>8</sup> on the basis of the following criteria: they were yielding 80 or more nuts per palm per year; they were regular bearers; the bunches were well supported by the petioles; they were free from all diseases and pests; they were 35 or more years of age; they had all the typical characters of the West Coast Tall var. confirmed by the progeny test to make sure that no hybrid palm is selected as mother palm, and most importantly, they were surrounded by 80% or more root (wilt)-affected palms in advanced stage of the disease (Figure 2) and had shown negative reaction to root (wilt) disease in the sero-

diagnostic test at the time of selection<sup>9</sup>. These palms are being subjected to sero-diagnostic test<sup>10</sup> every year; the fact that so far they have not contracted the disease in spite of being under heavy disease pressure, confirms their tolerance to root (wilt) disease.

Soil samples were collected from these root (wilt)-tolerant coconut palms, two from each of the three districts, viz. Alappuzha, Pathanamthitta and Kottayam in Kerala. Rhizosphere soils from diseased and healthy trees (approximately 3 kg) were collected from three sites at 1 m circumference of the palm, where active roots are located, and at two depths (0–25 and 25–50 cm). They were packed in clean plastic bags and stored at 4°C for further studies. Ten grams of moist soil was drawn from each sample and analysed for general microbial community, namely bacteria, actinomycetes and fungi and function-specific microbial community, namely free-living  $N_2$ -fixers, phosphate solubilizers, cellulose degraders and silicate solubilizers. Enumeration was done by serial dilution and pour plate method. Nutrient agar medium for bacteria<sup>11</sup>, Martin's Rose Bengal agar medium<sup>12</sup> for fungi, Ken Knight and Munaier's agar medium for actinomycetes<sup>11</sup>, N-free agar medium<sup>13</sup> for free-living  $N_2$ -fixers, Pikovskaya agar medium<sup>14</sup> for phosphate solubilizing bacteria, Czapek Dox cellulose agar medium<sup>15</sup> for cellulose degraders and modified Bunt and Rovira medium<sup>16</sup> for silicate solubilizers were used in this study and microbial population was expressed in terms of cfu per g of dry soil.

The counts of the culturable bacteria were significantly higher in the rhizosphere of the root (wilt)-diseased palms than those of the field-tolerant ones (Table 1). This difference was observed to be conspicuous at 0–25 cm depth. In diseased palms, the count was significantly higher at the upper level, whereas in field-tolerant palms, the bacterial count at both the depths was on par. The overall fungal count in both types of palms was not significantly different. However, the fungal count at 0–25 cm depth was higher in the case of tolerant palms compared to the diseased palms. The actinomycetes count was significantly higher in the rhizosphere of field-tolerant palms compared to root (wilt)-diseased palms at both the soil depths. The population recorded was significantly higher at 0–25 cm depth in both the cases. Presence of high bacterial count in the rhizosphere of root (wilt)-diseased palm has been well-documented earlier<sup>17–19</sup>. However, higher populations of actinomycetes and fungi (at 0–25 cm depth) in field-tolerant palms were contrary to the observations made by Potty *et al.*<sup>17</sup> and Alice *et al.*<sup>18</sup>.

In the function-specific microbial community (Table 2), the difference between field-tolerant and diseased palms, between the two depths, and the interactions for  $N_2$ -fixers were found to be significant. Disease-tolerant palms, on an average, recorded higher free-living nitrogen fixers compared to diseased palms. In addition, the  $N_2$ -fixers from the rhizosphere of field-tolerant palms produced higher amounts of polysaccharides on the growth medium.



**Figure 1.** A healthy coconut palm (*a*) and a root (wilt)-diseased coconut palm (*b*) (West Coast Tall variety).



**Figure 2.** A field-tolerant coconut palm growing amidst heavily diseased root (wilt)-affected palms (shown by arrows).

The phosphate solubilizing microbes were found to be on par in both types of palms, their population being higher at 25–50 cm depth of the soil. Preponderance of free-living  $N_2$ -fixers in the rhizosphere of field-tolerant palms and similarity in phosphate solubilizers population in both types of palms, corroborate the observations made earlier by Potty *et al.*<sup>17</sup> in their studies on changes in soil

microflora of root (wilt)-diseased and healthy palms as influenced by growing Napier grass and fertilizer application. Numerically, though the phosphate solubilizers were on par in both types of palms, it was observed that fungi like *Aspergillus niger*, *Aspergillus awamori*, *Aspergillus* spp. and *Penicillium* spp. were more in the rhizosphere of field-tolerant palms (Table 3). On an average, these fungi

**Table 1.** Population estimation of general microbial communities in rhizosphere of root (wilt)-diseased and field-tolerant coconut palms of WCT var. (results are an average of five replicates of each soil sample)

Soil sample	Bacteria ( $\times 10^5$ )	Actinomycetes ( $\times 10^2$ )	Fungi ( $\times 10^2$ )
Root (wilt) diseased palms (RWD)			
0–25 cm	10.53	14.80	5.31
25–50 cm	6.16	9.81	4.56
Field-tolerant palms (FTP)			
0–25 cm	4.80	27.18	8.55
25–50 cm	5.53	17.82	3.56
Mean RWD	8.35	12.31	4.88
Mean FTP	5.17	22.51	6.05
CD ( $P = 0.05$ ) RWD vs FTP	1.31	4.10	NS
CD ( $P = 0.05$ ) 0–25 vs 25–50 cm	1.31	4.10	1.62
CD ( $P = 0.05$ ) interaction	1.85	NS	2.29

**Table 2.** Population estimation of function-specific microbial communities in rhizosphere of root (wilt)-diseased and field-tolerant coconut palms of WCT var. (results are an average of five replicates of each soil sample)

Soil sample	Nitrogen-fixers ( $\times 10^2$ )	P-solubilizers ( $\times 10^2$ )	Cellulose degraders ( $\times 10^2$ )	Silicate solubilizers ( $\times 10^2$ )
Root (wilt) diseased palms (RWD)				
0–25 cm	21.68	2.81	2.75	0.80
25–50 cm	14.95	8.41	1.43	1.04
Field-tolerant palms (FTP)				
0–25 cm	73.40	4.23	3.23	5.03
25–50 cm	49.24	10.20	1.32	1.96
Mean RWD	18.31	5.61	2.09	0.92
Mean FTP	61.32	7.2	2.27	3.49
CD ( $P = 0.05$ ) RWD vs FTP	1.31	4.10	NS	0.63
CD ( $P = 0.05$ ) 0–25 vs 25–50 cm	1.31	4.10	1.62	0.63
CD ( $P = 0.05$ ) interaction	1.85	NS	2.29	0.89

**Table 3.** Distribution of microorganisms in rhizosphere of root (wilt)-diseased and field-tolerant coconut palms and their capacity for phosphate solubilization in Pikovskaya agar medium

Soil	Distribution of microbes (%)	Range of zone clearing (mm)
Root (wilt) diseased palms		
Bacteria	70	0.5–2.0
Actinomycetes	10	0.2–1.0
Fungi	20	1.0–7.0
Field-tolerant palms		
Bacteria	50	0.5–2.0
Actinomycetes	10	0.5–2.0
Fungi	40	2.0–7.0

were observed to produce clearing zones of 2–7 mm, while the bacteria (*Bacillus* spp. and *Pseudomonas* spp.) produced maximum clearing zones of about 2 mm; the size of the clearing zones being an indication of efficiency in phosphorus solubilization.

The cellulose degraders were also recorded to be on par in the rhizosphere of field-tolerant and diseased palms.

However, their numbers in both the cases were significantly higher at the top 0–25 cm of the soil. The population of cellulose degraders was not significantly different, although it could have been higher in the root (wilt)-diseased palms, since there are reports of roots getting rotten and their debris sloughed-off into the rhizosphere<sup>20</sup>. However, in later detailed studies, it was reported that root rotting was not seen in all the diseased palms<sup>21</sup> and was not considered as a characteristic symptom associated with root (wilt)-disease<sup>22</sup>. In the light of these findings, our observation seems to be in place with regard to population of cellulose degraders.

A key observation emerging from the data is the presence of significantly higher population of silicate solubilizing bacteria in the rhizosphere of field-tolerant palms at 0–25 cm depth compared to root (wilt)-diseased palms. The numbers of these function-specific bacteria were on par at 25–50 cm depth in both types of palms. This observation is of interest in the sense that it might have implications in nutrition, and thus the physical appearance of field tolerant *vis-à-vis* diseased palms. Majority of the silicate solubilizers encountered in our study were *Bacillus* spp. and *Pseudomo-*



*nas* spp., though at times *Aspergillus* sp. and *Penicillium* sp. were also identified.

Though the general bacterial population was high in the root zone of the root (wilt)-diseased palms compared to field-tolerant palms, function-specific microbes, viz. nitrogen-fixers, phosphate solubilizers, cellulose degraders and silicate solubilizers, which are mostly bacteria, are present in significantly higher numbers in the rhizosphere of field-tolerant palms than in diseased palms. Careful interpretation revealed that as high as 3.6% of the total bacterial population was made up of function-specific bacteria in disease-tolerant palms compared to the mere 0.21% in diseased palms. Presence of these function-specific microbes could be playing a role in improving the nutrient availability to the palms, thereby perhaps conferring vigour to the field-tolerant palms.

Silica is primarily deposited on the walls of epidermis and vascular tissues and confers strength and rigidity to the leaves, more particularly so in monocotyledonous coconut crop. In a study at the Central Plantation Crops Research Institute at Kasaragod, on nutritional management of root (wilt)-affected adult coconut palms with organic manure and silicon during 1995–98, it was reported that the silicon dioxide content in the leaf samples (14th leaf) of root (wilt)-diseased palm<sup>23</sup> was consistently found to be around 0.24 to 1.7%, which was significantly lower compared to the leaf of healthy coconut palm<sup>24</sup> containing SiO<sub>2</sub> to the tune of 59–134%. In this context, it is important to note that field-tolerant coconut palms exhibit normal, erect leaves with the leaflets avoiding bending and having abundant chlorophyll content, while diseased palms exhibit wilting and drooping of leaves, flaccidity, ribbing with yellowing and necrosis of leaflets<sup>25</sup>.

Though the soils of Kerala, especially the sandy and sandy loams have high silica content<sup>26</sup>, the soil silicate is generally present in unavailable polymerized forms and for its absorption by plants, it has to be depolymerized and rendered soluble by means of biological or chemical reactions. A variety of soil microorganisms have been found to solubilize silicates<sup>16,27</sup>. These bacteria solubilize the insoluble silicates by production of CO<sub>2</sub>, organic acids and/or exopolysaccharides. Gluconate-promoted dissolution of silicates like albite, quartz and kaolinite by sub-surface bacteria has been recorded earlier<sup>28</sup>. Large numbers of silicate-solubilizing bacteria in the root zones of field-tolerant palms observed in this study could improve the available pool of silica (silicic acid, H<sub>4</sub>SiO<sub>4</sub>) to be absorbed by these palms, which is reflected in their firm and erect leaves arranged to receive maximum sunlight. Field-tolerant palms are shown to have better photosynthetic turnover<sup>29</sup>, perhaps by virtue of higher silica uptake, since silica crystals are implicated in absorbing optimum photo energy for efficient photosynthetic process in many crops, including monocots<sup>30</sup>. Physiologically, presence of silica in leaf epidermis provides mechanical strength to the stomata and regulates transpiration rate. Less numbers of silicate

solubilizers in root (wilt)-diseased palms could possibly be one of the reasons for abnormal stomatal activity permitting higher transpiration in such palms<sup>31,32</sup>. Absence of sufficient silica cells in the leaves of diseased palms could cause the stomata to collapse and remain abnormally open.

The vectors of root (wilt) disease, *S. typica* and *P. moesta*, introduce the phytoplasma into the palms by inserting the stylet through the stomatal opening present in the lower epidermis. Here too, the presence of more silica cells in field-tolerant palms may provide the leaves with higher mechanical and tensile strength, and prevent the vectors from depositing the pathogen, even though they have been observed to colonize healthy palms<sup>4</sup>. Another physiological condition is the presence of high levels of free phenols in the roots of healthy palms compared to root (wilt)-diseased palms<sup>33</sup>. There is evidence to show that more silica in roots promotes more phenol production<sup>34</sup>. Also, presence of silica in many monocot and vegetable crops provides resistance to fungal diseases<sup>35</sup>. In coconut too, high silica content in field-tolerant palms might help them evade secondary fungal infection, whereas root (wilt)-diseased palms succumb to leaf rot disease caused by a fungal complex.

The rhizosphere microbial make-up of any plant is dependent upon the rhizodeposition (organic acids and other exudates produced by the roots), which is again dependent on the genetics of the plant, its health, soil type and anthropogenic interventions. With the above observations of differences in the rhizosphere microbial ecology of root (wilt)-diseased and field-tolerant coconut palms, we would like to hypothesize that the presence of more number of silicate-solubilizing bacteria in the rhizosphere of field-tolerant coconut palms could promote more silica uptake, which may provide tensile strength to the leaves to resist vector feeding and prevent phytoplasma inoculation.

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## IWAVE: an ocean simulation model for internal waves

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**Effective usage of scarce resources (ships, instruments, etc.) requires a coherent and rational approach to develop any underwater system. One of the important tasks towards achieving this is to simulate the performance of the proposed system under all oceanic conditions before it is being developed. Internal waves (IW) play a major role in ocean thermodynamics, underwater acoustic transmission, etc. In order to understand the behaviour of IWs and their role in various physical processes in the ocean, we have developed an IW model, IWAVE based on the Garrett–Munk modal spectrum. In this communication we present detailed implementation procedures of the model and also a technique to solve the eigenvalue problem accurately. An algorithm based on central finite difference and QR techniques was implemented to find the eigen-wave-numbers and modes (dispersion relations). IWAVE simulates temperature and salinity (estimated sound speed also) structure due to IWs in the ocean. Experiments were conducted to validate the model off the west coast of India. Variances of the measured and simulated sound speed are in good agreement.**

**Keywords:** Dispersion relation, GM model, internal waves, simulation, validation.

IN a stratified medium, a fluid parcel displaced from its equilibrium level experiences a restoring force propor-

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