https://doi.org/10.29321/MAJ.10.A03557

Studies on Wilt Disease of Betelvine

by

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Introduction: The wilt disease on betelvine (*Piper betle* L.) is widespread in many parts of Madras State, especially in the districts of Salem, Tiruchirappalli and Coimbatore causing heavy losses to the crop. Ever since the discovery of this disease by Dastur (1935) from Madhya Pradesh, as due to the fungus *Phytophthora parasitica* Dast. it has received attention of scientists all over India and other countries like Malaya, Burma, Pakistan, etc. Mahmud (1952) has reviewed much of the literature on this disease. A perusal of this indicates that the work so far has been largely confined to the control of the disease and little is known about its etiology and the behaviour of the pathogen. The present report is the results of studies conducted on these aspects.

Material and Methods: *Isolation of the Fungus:* The fungus *Phytophthora* was isolated from wilted betelvine plants of *Karpuri* variety from Pothanur (Salem District) by planting pieces of infected stem on oat agar. The stem pieces were taken from the places where the healthy and diseased portion meet. The fungus was purified by single sporangium isolation and maintained on oat agar slants and sub-cultured once in three weeks.

Method of inoculation: In pot culture studies the inoculum was multiplied on sand-maize medium (9:1) in narrow mouthed 24 oz bottles and used for inoculation. In all the studies conducted in pots sterilized soil was used. Betelvine, *Karpuri* variety was used in all studies. Earthen pots of 9" diameter were used throughout the studies.

Results: (1) *Pathogen:* The pathogen was identified as *Phytophthora parasitica* Dast. It produced a white fluffy growth on oat agar medium. Sporangia were produced when the mycelium was suspended in non-sterile well water, but not on oat agar. Sporangia were mostly pear shaped, spherical or oval with prominent papilla and measured 21.6μ to 90.8$\mu \times 19.2\mu - 69.2\mu$. They germinated directly by producing germ tubes and under favourable conditions produced zoospores.

(2) *Pathogenicity:* The pathogenicity of the fungus was established by inoculating 20-day old fungus inoculum on rooted cuttings of betelvine in pots. The plants died in 5 to 17 days after inoculation.

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Received on 15-7-1968.

(3) *Symptomatology and etiology:* The characteristic symptom noticed in infected betelvine plants was the general loss of lustre and drooping of the leaves and tender shoots. Thereafter the plants wilted completely within two to three days. Wilting usually started from the top and proceeded downwards. When the plants were pulled out at the earliest stages of wilting, no rot of the underground portions of the stem was seen. In a longitudinal section of the wilted vines, a dark brown discoloration of the xylem tissue both above and below the collar region was seen (Plate I, fig. 2). In advanced stages of wilting the underground portions of stem and roots were found to rot. The above symptoms indicated that the disease is probably a vascular wilt rather than a root or stem rot.

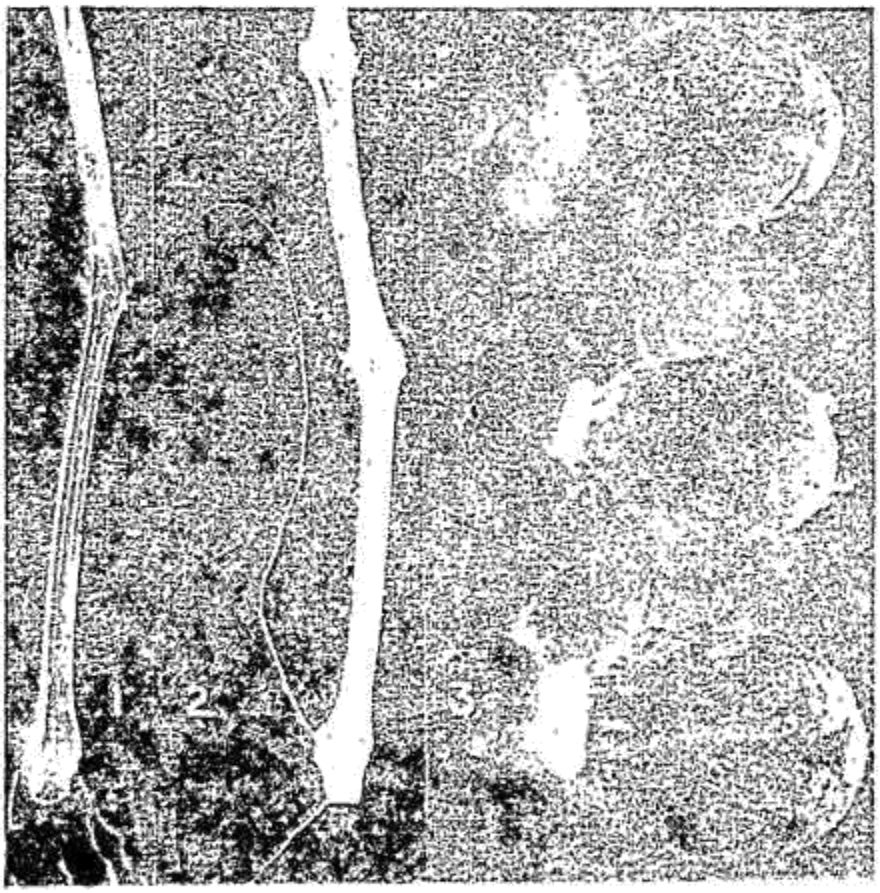

PLATE 1

1. Wilted betelvine stem longitudinally split to show the vascular browning.
2. Healthy stem similarly split.
3. Castor seed floated in water after incubation in *Phytophthora* inoculated soil. Note the growth of the fungus from the aril.

The culture filtrate was examined for pectic enzymes. The fungus was grown in three different media, *viz.*, dextrose pectin medium, asparagine pectin medium and Leonian's medium, and the culture filtrate tested for pectic

enzymes by the potato disc method. The discs were not macerated even after prolonged treatment. The culture filtrate was then examined for the presence of any wilt causing agents. The fungus was grown in Leonian's medium for 22 days in 250 ml. flasks, and the culture filtrate obtained by filtering through Whatman No. 1 filter paper and then passing through a sterilized bacteriological filter. The filtrate was used as such and at different dilutions with sterile distilled water to give concentrations of 1 in 20, 1 in 40, 1 in 80 and 1 in 100. The uninoculated medium and distilled water were kept as controls. The effect of these treatments on betelvine cuttings and tomato seedlings was tested in sterile pyrex tubes. The experiment was under observation for 72 hours. The extent of vascular browning in betelvine cuttings is presented in Table 1.

TABLE 1. *Effect of culture filtrate on betelvine stem cuttings — var. Karpuri (Medium : Leonian's)*

Concentration	Vascular browning - average ht. in cm.
Culture filtrate (pH 4.5)	
(a) Full	4.75
(b) 1 in 20	1 2
(c) 1 in 40	0.3
(d) 1 in 80	0.0
(e) 1 in 100	0.0
Uninoculated medium (pH 4.4)	0.0
Distilled water	0.0

0.0 — No Browning

The results obtained showed that the culture filtrate was toxic and caused vascular browning in betelvine cuttings and tomato seedlings. The toxic effect in both cases was reduced on progressive dilutions. Tomato seedlings showed severe crinkling and chlorosis of leaves in the neat culture filtrate. The experiment was repeated by using modified Leonian's medium with asparagine 0.05%, thiamine 0.01% and sucrose 0.25% or maltose 0.25%. The culture filtrate was dialized for 24 hours under tap water, and tested for toxicity using tomato seedlings. The tomato seedlings showed symptoms of toxicity in the culture filtrate as before.

(4) *Saprophytic status of the fungus in the soil:* (a) The ability of the fungus to colonise dead organic matter in soil was studied. Inoculum of *Phytophthora parasitica* was incorporated at the rate of 10 per cent by weight in sterile and non-sterile soil in pots. Twenty sterilized betelvine stem pieces (3" length) were buried in each pot. After 72 hours the stem pieces

were taken out washed free of soil, surface sterilized with calcium hyphochlorate solution (1 in 14 concentration) for 1 minute and then washed several times in sterile distilled water. These pieces were then suspended in sterile water to recover the fungus. The pieces incubated in non-sterile soil did not yield any fungus. In the case of sterile soil, however, 20 per cent of the pieces showed the growth of the fungus.

(b) *Recovery of the fungus by using castor seed:* To unsterilized soil in pots, 10 per cent of the fungus inoculum was added and mixed. Castor seeds (*Ricinus communis* L.) 25 in number were surface sterilized in 1 per cent mercuric chloride for a few seconds, washed in sterile water and then sown in the above soil. After 63 hours incubation, the seeds were removed, surface sterilized and then suspended in sterile water. Seventy six per cent of the seeds showed fungal growth. The fungus was found to grow on the aril of the castor seed near the micropylar end. (Plate 1, fig. 3). The identity of the fungus was determined by transferring the growth to non-sterile well water in which sporangia were produced.

(c) *Optimum incubation period and optimum inoculum dosage for recovery of the fungus on castor seeds:* Castor seeds were incubated for 2, 4, 8, 24, 48, 72 and 90 hours in *Phytophthora* inoculated non-sterile soil. Ten seeds were used per treatment. Table 2 gives the results of this experiment.

TABLE 2. *Recovery of the fungus under different incubation periods*

Particulars	Period of incubation (in hours)							Control
	2	4	8	24	48	72	96	
No. of seeds (Castor TMV. 1) sown	10	10	10	10	10	10	10	10
No. on which fungus was recovered	—	—	—	4	3	9	8	—
Percentage of recovery	—	—	—	40%	30%	90%	80%	—

Percentage of inoculum used : 10%

From the results above it could be seen that minimum incubation period required was 24 hours and the maximum 72 hours. The fungus did not colonize the seeds up to 8 hours.

As before the experiment was repeated by using 1%, 5%, 10%, 20%, 40%, 60%, 80% and 100% inoculum doses using 10 seeds in each and the seeds removed after 72 hours incubation period. (Vide Table 3).

TABLE 3. *Recovery of fungus under different inoculum dosages*

Particulars	Inoculum doses (%)								Control
	1	5	10	20	40	60	80	100	
No. of castor seeds sown	10	10	10	10	10	10	10	10	10
No. in which fungus was recovered	8	9	7	6	7	9	9	10	—
Percentage of infection	80	90	70	60	70	90	90	100	—

The castor seed technique appears to be sensitive as it could detect an inoculum dosage as low as 1 per cent.

(d) *Isolation of the pathogen from infected betelvine garden soil and irrigation water using castor seeds:* At the Betelvine Research Station, Pothanur, castor seeds were buried in the soil at 4-6" depth in the control as well as fungicide treated plots. The fungicides used were Bordeaux mixture 1% and copper oxychloride 0 25%. Sixty three per cent of the seeds from untreated plots and fifty per cent from the treated plots, yielded the fungus

Castor seeds were put in small coarse cloth bags and tied well and then suspended in the irrigation water flowing in the trenches in between the rows of betelvine plants. Sixty three per cent of the seeds removed after 42 hours yielded the fungus.

Discussion: The wilt disease of betelvine was first reported by Dastur (1935) who identified the causal agent as *Phytophthora parasitica* Dast. This is confirmed in the present studies. The etiology of the disease is not well understood. It is clear from the present results that the disease is not a root or stem rot. The rotting of the underground part of the stem and roots in late stages of the disease appears to be due to saprophytic invasion. Vascular browning far in advance of the site of infection strongly suggests a vascular wilt syndrome. The presence of a vascular browning agent in the culture filtrate of the pathogen and the absence of pectin destroying enzymes lends strong support to this view. It may be pointed out here that a wilt toxin has been demonstrated in *Phytophthora parasitica* var. *nicotianae*, the tobacco black shank pathogen (Walf, 1953). *P. parasitica* was unable to colonise dead organic matter in unsterilised soil. It was however able to colonise a live bait like castor seed. The fungus appears therefore to be a 'root inhabitant' rather than a 'soil inhabitant'. Garrett (1956) states that specialised pathogens are unable to colonise dead organic matter, presumably because parasitic specialisation has unfitted them for saprophytic competition. The castor seed

technique appears to be a sensitive and useful technique to demonstrate the presence and activity of the fungus in soil. Using this technique the abundant presence of *P. parasitica* in soils and irrigation water of betelvine gardens has been demonstrated in the present study. Such "baiting" techniques, using apple and lemon fruits for the isolation of *Phytophthora* spp. have been employed earlier by Campbell (1947) and Klotz *et al* (1959).

Summary: The pathogen causing wilt disease of betelvine was isolated and identified as *Phytophthora parasitica* Dast. The disease appears to be a vascular wilt and not a root rot. Extensive vascular browning was observed in wilted plants. No pectin destroying enzymes could be demonstrated in the culture filtrate of the fungus but a vascular browning agent was present. The fungus could be easily isolated from soil and irrigation water using live castor seeds as baits. It did not colonize dead betelvine stem pieces in non sterile soil.

Acknowledgement: The authors are grateful to the University of Madras for granting permission to publish this paper which forms part of the M.Sc.(Ag.) dissertation of the senior author.

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