QUICK WILT DISEASE OF PEPPER-II

THE TECHNIQUES FOR SCREENING PEPPER VARIETIES AGAINST QUICK WILT DISEASE CAUSED BY PHYTOPHTHORA PALMIVORA (BUTLER) BUTLER

By

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THESIS

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DEDICATED TO
THE LOVING MEMORY
OF
MY MOTHER

DECLARATION

I hereby declare that this thesis entitled "Quick wilt disease of Pepper-II. The techniques for screening pepper varieties against quick wilt disease caused by *Phytophthora palmivora* (Butler) Butler" is a bonafide record of work done by me during the course of research work and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis is a record of research work done independently by Kumari T.N. Vilasini under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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1. Symptom expression on the leaves of *Piper nigrum* (Panniyur-1)

Introduction

INTRODUCTION

The quick wilt (foot rot) disease of black pepper (*Piper nigrum* Lin.) caused by the fungal pathogen *Phytophthora palmivora* (Butler) Butler is a serious malady wherever the crop is cultivated. At present Kerala contributes 96 per cent of the production of pepper in the country. One of the many reasons for the low productivity of pepper vines in Kerala (0.227 kg/vine/annum) is the occurrence of this disease which causes partial or complete destruction of the vines. The impact of the disease on the agricultural economy of the state is substantial and export earnings are considerably reduced due to this devastating disease.

Concerted efforts have been made in the past in the pepper growing countries to study the various aspects of the disease and thereby to control it effectively. Despite all such efforts, effective and economical control measures have not been formulated so far. The disease is

characterised by rapid development leading to plant mortality and the infection of sub-terranean tissues. This nature of the disease and the high rain fall associated with the out-break of the disease make the control measures ineffective.

Identification of resistant genotypes and development of improved types incorporating the factor(s) of resistance would, therefore, be the most desirable strategy for containing the disease. In black pepper which is highly heterozygous, the scope for generating further genetic variability by open pollination and hybridisation is considerable and there is tremendous potential for exploitation of planned crossings for development of resistance to diseases.

Conducting studies on the resistance spectrum of the types including wild or semi-wild types occurring in the native habitates of the crop is hence of great value as a forerunner of further studies on this aspect. The centre of origin of the crop is the evergreen tracts of western ghats of Kerala (Gamble, 1918; Purseglove, 1968). A large collection of wild types from this

area has been made and these are maintained as part of the germplasm reserve of the Pepper Research Scheme at Vellanikkara. In order to screen these materials for resistance to the wilt disease a rapid and most effective method has to be developed.

The greatest difficulty in screening the plants under natural conditions is that the inoculation with the pathogen will be effective only during a very short period of the year, that is, the monsoon period (July-August) when the atmospheric temperature and humidity levels are most conducive for disease development. This imposes limits to the time available for large scale screening of pepper types against the disease. To tide over this difficulty, a technique which can be adopted throughout the year has to be evolved and standardised. The present study was therefore undertaken with this major objective.

The study consists of the following aspects:-

1. Study of the disease symptoms after inoculation of one year old cuttings of pepper adopting conventional methods.

2. Recording of the development of necrotic lesions on the roots after dipping the root system of rooted cuttings in standarised zoospore suspension.

3. Study of the symptom development on the roots after dipping the cuttings in partially purified culture filtrate devoid of propagules but containing the toxic metabolite.

4. Treatment of cut ends of stem portions in partially purified culture filtrate as mentioned in three above.

5. Comparative evaluation of the above methods to ascertain the efficacy of the different methods.

Review Of Literature

REVIEW OF LITERATURE

Pepper (*Piper nigrum* Lin.) is susceptible to many fungal and bacterial diseases. Among the diseases, the quick wilt (also called as foot rot) caused by *Phytophthora palmivora* (Butler) Butler is the most serious in all the pepper growing tracts of the world. According to the reports from different pepper growing tracts of the world, the losses in yield of pepper and death of vine due to this devastating disease range from 10 to 50 per cent. Leefman (1934) reported that in West Borneo the crop suffered from the foot rot disease due to the attack of *Phytophthora* resulting in 10 per cent loss in yield. Holliday and Mowat (1961) estimated that losses due to foot rot had amounted to about 7,000 tonnes in Sarawak. Samraj and Jose (1966) estimated that 20 per cent of the vine was destroyed by this disease in India. According to Albuquerque (1966), occurrence of the disease in the Amazon region destroyed 1,000,000 plants and led to a loss of more than 3,000 tonnes of black pepper. Harper (1974) reported an yield loss of 50 per cent in Indonesia, due to this disease.

The incidence of the wilt disease of black pepper in India was reported in the early part of twentieth Century (Barber, 1902; 1903 and 1905). Butler (1906) reported that the pepper vine wilt was a serious disease in Wynad area of Kerala. Later, he investigated the disease in this area and had partially described the symptomatology. According to him, the first visible symptom was the starved appearance of the vines and this was attributed to the loss of rigidity in the leaves and leaf stalks resulting in their drooping. The affected portion of the climbing stem was found to fall away from the standard causing sudden death of the vine (Butler, 1906 and 1918).

Venkata Rao (1929) first established the association of Phytophthora sp. with the wilt of P. nigrum in Mysore. Leefman (1934) also reported that foot rot of P. nigrum was caused by a species of Phytophthora.

Muller (1936) observed a similar type of disease from Dutch East Indies, caused by Phytophthora palmivora var. piperis. He has made a detailed study of the disease and described the symptomatology and suggested control measures. According to him,

all parts of the plant at all stages of growth are susceptible to the disease. Three types of symptoms, namely, leaf rot, collar rot and root rot are generally observed in the field. He also observed greyish-brown spots of 5 mm diameter near the tips and margins of the leaves surrounded by a zone of 3 to 5 mm with an yellowish fluid exuding from the underside of the affected tissues. Thereafter the leaves turned yellow, wilted and dropped. The first symptom is a blackening of parts of the lower leaves near the soil surface (Cook, 1960). Samraj and Jose (1966) observed small, irregular, black patches on leaves which rapidly enlarged in size and covered the entire area.

Holliday and Mowat (1963) gave a detailed description of the symptoms. According to them, infected leaves are found on the lower part of the foliage and usually within a few centimeters of the surface of the mound. A necrotic lesion develops which, if in the centre of the leaf, is circular. The lesion may sometimes be at the edge of the lamina and more usually at the tip where water drop collects. Under favourable conditions, the deep brown lesion spreads uniformly and in transmitted

light, its edge is seen to be fimbriate. They also stated that an infected leaf has more than one lesion and several scattered circular areas are the result of multiple infection. Before all the lamina becomes necrotic, the leaf abscisses. After development of necrotic spots, if unfavourable conditions prevail, the spread of the lesion ceases or one or two vaguely defined concentric markings are left on the surface.

Turner (1969) observed that on artificial inoculation on leaves of P. nigrum, visible lesions developed within 24 to 36 hours on immature leaves and 36 to 48 hours on mature leaves. Lesions expanded subsequently and expansion parellel to veins tended to be more rapid along the veins than in the tissue between. Under high humidity condition, chocolate-brown lesions with the typically fringed margin developed. In alternate wet and dry conditions, distinct concentric zonations occurred. There was no difference between lesion development on detached and attached leaves.

Nambiar and Sharma (1976) observed water soaked lesions with fimbriate margin as the initial symptom

which may appear on the leaves starting from tip, base of the lamina, leaf margin or from centre. They also found concentric rings on fully developed lesions and varying degrees of defoliation.

Mammootty *et al.* (1980) reported that the first visible symptom on the leaves was pale coloured, water soaked regions in the infection court. This region later turned to light brown and then to dark brown within one or two days and the infected region showed signs of wilting. The rotting was rapid and it covered the entire leaf area within a short period. The fully developed lesion had a holonecrotic centre surrounded by a brown plesionecrotic zone which in turn was surrounded by yellow halo. Zonations were noticed under alternate dry and wet weather conditions.

The disease was more lethal when the pathogen attacked the collar region of the plant. Muller (1936) reported that the diseased cortex turned dark watery-green to black. He also observed that external symptoms were visible only after the complete disintegration of internal tissues. In severe cases the bark often peeled off and the centre split into a bundle of xylem vessels.

Samraj and Jose (1966) observed that the infection occurred at a height of 25 cm above the soil level. They also found that the tissues at the affected region became soft and decayed. The leaves turned pale and flaccid and ultimately the plant died. Lee (1973) observed vascular browning at the points beyond the site of infection. The infected stem at ground level caused rotting and death of the vines in two to three weeks (Nambiar and Sharma, 1976). They also observed discolouration of the infected region which emitted a noxious odour. The necrosis progressed downwards to the below ground portion of the stem and then to the root system.

Mammootty et al. (1980) observed that the initial symptom on the stem was the appearance of a water soaked region. This water soaked region turned black brown within two to three days. Within three to six days, flaccidity of younger leaves occurred followed by yellowing of younger leaves and flaccidity of the mature leaves. When rotting of the internal tissues became severe, the leaves shed and twigs either got separated or dried above the

infected region. They also observed that vascular discolouration up to a height of 100 cm beyond the point of infection. Death of the vine takes place within 12 to 20 days.

Butler (1906) observed blackening of the roots which extended into the base of the stem. Holliday and Mowat (1957, 1963) reported that the infection started from the fine roots and the cortical necrosis spread from the main roots to the underground stem, after which symptoms are seen above ground.

Alconero et al. (1972) noticed root infection, 24 to 48 hours after artificial inoculation. Root tips were darkened and the infection spread acropetally. Roots decayed completely and shoots wilted within four days.

Mammootty et al. (1980) found that the brown discolouration of the fine roots was the initial symptom which gradually spread upwards affecting branches and main roots within a few days. Discolouration of the vascular tissue was observed up to a distance of 25 cms away from the point of infection. When the main branches of the roots

and the underground stem got infected, the detectable symptoms on the stem and leaves were noticed. The first symptom on the aerial part due to root infection was similar to that of vine infection.

Holliday and Mowat (1963) made an attempt to study the resistance of the pepper clones, four from India (Balankotta, Cheriyakanjakadan, Kalluvalli, Uthiramkotta), three from Indonesia (Belantung, Banka, Djambi), two from Sarawak (Kuching, Sarikei) using different screening techniques. They tried zoospore suspension for inoculating the roots, zoospore suspension and culture disc for inoculating the leaves and pot test for resistance. Single noded rooted clonal cuttings were used for inoculating the roots. The cuttings were carefully uprooted, washed thoroughly and placed in the plastic dishes containing zoospore suspension. The entire roots were immersed in zoospore suspension for 24 hours and then transferred to the nutrient solution. The degree of necrosis was assessed on each cutting and scored as follows:

Degree of necrosis	Score
a. More than ¾ roots necrotic	10
b. ¼ to ¾ roots necrotic	5
c. Less than ¼ roots necrotic	2
d. No necrosis	0

The assessment was made six days after removal from the inoculum. A clone, therefore, where each of the ten cuttings had ¼ of the roots necrotic would score 100 per cent susceptibility. None of the clones showed immunity and the sporangia developed on the necrotic area. They reported that all the four Indian clones and two of the Indonesian ones (Djambi and Belantung) showed appreciable resistance. The resistance was apparently manifested by the rate at which necrosis spread in the roots. The two Sarawak clones, Kuching and Sarikei and the Indonesian variety, Banka were found to be more susceptible than the remaining ones. They inoculated the detached unwounded mature and immature leaves and detached wounded mature leaves. For immature leaves, they used zoospore suspension at the rate of 0.06 ml for each inoculation point and for mature leaves 1 cm diameter oat meal agar culture disc was placed. After placing in Petri's solution for three days, the leaves were wounded by single needle puncturing. In all leaves, they inoculated only the lower surface. The degree of infection was assessed four days after

applying the inoculum as follows:

Degree of infection	Score
a. Fast growing lesion	10
b. Clear lesion but develops late and slowly	5
c. Necrotic speckling, no marked lesion	2
d. No necrosis	0

The results were expressed on a percentage basis. The results of leaf inoculation showed a general agreement with the root inoculation. The mature leaves showed higher resistance. Of the Indian clones, Balankotta and Cheriyakaniakadan were the best. Djambi and Belantung from Indonesia were equally good. The young leaves of the other two varieties from India were rather susceptible to infection. Here also Kuching, Sarikei and Banks showed significantly higher susceptibility ratings. But Sarikei exhibited high resistance of intact mature leaves.

In the pot test for resistance, they used all the above varieties except Sarikei. The rooted cuttings were potted in partially sterilised sand mixture. After six months of growth, the plants were tested by using high and low inoculum levels. The rate of wilting was scored in each variety.

The susceptible variety took 12 days for wilting while the resistant one took 19 days. There was a significant difference between the very susceptible Kuching and Banka (100.0 and 93.7 per cent, wilt, respectively) and remaining six clones (19.3 to 0.0 per cent wilt) showed some resistance. None of the cuttings of Uthiramkotta showed wilting. There was no significant difference between the high and low inoculum levels.

Ruppel and Almeyda (1965) tested six Piper species, namely, P. aduncum, P. blatterum, P. citrifolium, P. scabrum, P. trelawneanum and P. nigrum against the collar rot incited by Phytophthora in order to determine the suitability of native Piper species as root stock in Puerto Rico. They used the stem wounding inoculation technique for testing the susceptibility of Piper species. Stems of each species were wounded with a sterile scalpel three to five cm above the soil level and mycelium from one week old potato dextrose agar culture was inserted into the wounds and covered with anhydrous lanolin to prevent desication. Thirty days after inoculation, the plants were examined for external symptoms and were split longitudinally to determine

the internal rot. They followed an arbitrary scale for rating disease intensity, namely, 0 = no disease; 1 = no external symptoms, internal discolouration 10 mm or less from inoculation wound; 2 = external necrosis around wound, internal discolouration 11-50 mm from inoculation wound; 3 = plants wilted and extensive external necrosis evident around wounds, internal discolouration extensive (up to 25 cm) and 4 = plants dead. They observed that all of the Piper spp. were susceptible to collar rot pathogen to varying degrees. In addition to P. nigrum, only P. blattarum and P. citrifolium exhibited high disease indices. The reaction of P. scabrum and P. trelease̊anum was uniformly low in all plants. P. aduncum showed a high degree of resistance to the collar rot pathogen.

Leather (1967) tested four black pepper varieties, Balankotta, Kalbalamkotta, Kalluvalli and Karinkotta and Piper aduncum. He used wound inoculation method of roots and stem. In roots, the fungus grown on oat meal agar was placed after slightly scratching the cortex. On the stems, shallow slits were made into the cortex and oat meal agar culture was placed. He found that all the

varieties of *P. nigrum* were susceptible to the wound inoculation and none of them produced the symptoms when the pathogen was inoculated in unwounded portion. *P. aduncum* was found to be apparently resistant.

Turner (1971) conducted a resistance test with *P. palmivora* isolated from *P. nigrum* on thirty two species of *Piper* and common weeds found in pepper gardens as alternate hosts of *P. palmivora*. For this test, he used root dip inoculation in zoospore suspension. Apart from root inoculation tests, unwounded, excised leaves of *Piper* and *Peperomia* were inoculated with 0.5 cm oat meal agar disc of the pathogen. The zoospore suspension was prepared from oat meal agar cultures grown in petri plates of 8.5 cm diameter and placed in plastic dishes and just covered with Petri's solution to induce the formation of sporangia, the solution being changed daily. After three days, the solution was replaced by distilled water at the rate of 250 ml/culture and kept over night at 18 to 22°C to induce germination. The next morning the resulting zoospore suspension was filtered through a single layer of muslin. The root system of the tested species was placed in the zoospore suspension. After 24 hours, the inoculum was replaced by nutrient solution and the plants

left for further six days. The roots were examined for necrosis and the production of sporangia by the pathogen. The ratings were as follows:

Root necrosis absent	-
Root necrosis less than 10%	+
Root necrosis 10 - 25%	+ +
Root necrosis 25 - 50%	+ + +
Root necrosis 50 - 75%	+ + + +
Root necrosis 75 -100%	+ + + + +
Sporangia present	+
Sporangia absent	-

This study revealed that none of the tested crop plants, weeds or *Peperomia* sp. took infection. In the *Piper* spp. tested, the amount of root necrosis varied with the species. Necrosis occurred in all species, with the exception of *P. colubrinum* and *P. obliquum* which were resistant. In *P. scabrum*, although necrosis occurred, sporangia could not be detected on the roots. All the *Piper* spp. from south-east Asia became infected but a good number of *Piper* species from Africa and South America were found to be resistant to leaf infection. All *Peperomia* species, tested were free from infection.

Alconero *et al.* (1972) tested four varieties of black pepper, Kudravaly, Kalluvalli, Kotanadan and

Uthiramkotta against *P. palmivora*. They used two methods for studying the root infection. In the first method, single node rooted cuttings were transplanted in clay pots filled with steamed soil. The inoculum was mixed with the soil at the rate of two and four per cent by weight as four weeks old corn meal sand culture of the pathogen.

In the second method, they tried four varieties of *P. nigrum*, Kalluvally, Kudravali, Balamkotta and Bangha. Apart from *P. nigrum*, they tried *P. colubrinum* also. One-noded cuttings were rooted in tap water in 125 ml flasks and then placed into 20 ml beakers filled with a water suspension of zoospores. Root samples of these treated plants were observed after 6, 9, 12, 24, 48 and 96 hours of immersion in zoospore suspension. Infection and development of the fungus in roots were studied. The infection on roots by zoospore suspension was first detected by superficial observation, 24 to 48 hours after immersion. Within four days, the roots were completely decayed and shoots wilted. They found that there was no difference in the degree of root infection among the black pepper varieties but *P. colubrinum* was found to be highly resistant to this pathogen.

For testing the stem infection, they used the above four *P. nigrum* varieties and *P. colubrinum*. Two different methods were tried for this also.

The cuttings were rooted in vermiculite and planted in steamed soil in 4" clay pots. The stems were wounded slightly with a sterile razor blade 10 cm from the soil level and a small piece of mycelium of the pathogen was introduced into the wound.

In the second method, the stem inoculations were made on the cuttings rooted in water.

Inoculation by wounding the stems resulted in rapid decay but this did not produce the wilt symptom in the field. The symptoms were expressed in 12 to 24 hours. No apparent differences in the resistance were observed among the black pepper varieties by the inoculation methods and all the tested black pepper varieties were found to be highly susceptible but *P. colubrinum* showed high resistance to infection of *P. palmivora*.

Turner (1973) tested the varietal reaction to the pathogen *P. palmivora* and found that Balankotta was the most resistant. But the other Indian

varieties like Cheriyakaniyakadan, Kalluvalli and Uthiramkotta were markedly more susceptible than Balankotta but these showed little difference between each other in susceptibility. The variety Kuching, a Malayasian type was highly susceptible.

A technique for screening black pepper (P. nigrum) with P. palmivora was developed by Sarma and Nambiar (1979). The following three different procedures of inoculation were adopted to screen black pepper varieties.

They found that the last procedure was most useful for rapid screening of black pepper varieties against P. palmivora.

1. Adding inoculum directly to the rooted cuttings raised in polythene bags.
2. Dipping the root system in inoculum for 10 minutes.
3. Keeping the rooted cuttings in inoculum for 48 hours and later transplanting.

Sarma et al. (1980) screened 41 cultivars of P. nigrum and 74 wild pepper types against P. palmivora adopting root dip inoculation method. After 48 hours of dipping, the cuttings were transplanted in soil and

kept at 25°C. The speed of mortality and percentage of infection based on number of wilted cuttings were recorded. They found that none of the cultivars and wild Piper spp. tested showed any reasonable degree of tolerance. The varieties Narayakodi, Kalluvalli, Uthirankotta and Balankotta showed low percentage of infection, while the cultivar Sullia was rated as highly susceptible.

Different techniques were used for screening other crops against Phytophthora diseases.

Demaree and Jeffers (1944) tested the resistance of strawberries to red stele (P. fragariae) using pot infection technique. The fungus was multiplied on Lima bean agar and inoculated in soil in the potted plants. The susceptible ones were eliminated and the resistant ones were reinoculated for further testing.

Busch (1949) reported artificial inoculation as a method of testing the resistance of 120 species and varieties of tomato against virulent strain of P. infestans (Mont.) de Bary. from tomato under green house conditions.

Bougnicourt (1950) developed a method for testing the resistance of Hevea rubber to P. palmivora. Mycelia bearing numerous sporangia were taken in a

Van Trighen Cell filled with sterile water and chilled for 30-40 minutes as a result of which zoospores were released. Vaseline was applied to the rim of the cell, which was then fixed against the trunk of the tree, so that the water containing the zoospores came in contact with the tissues. It was kept as such for three or four days. The relative resistance was determined by recording the degree of infection according to a pre-determined scale about one month after applying the cell. This method was also used to ascertain the resistance of Cinchona ledgeriana to a Phytophthora sp. causing a serious collar rot in Annam and Laos.

Hickman and Mary (1951) tested the resistance of straw berries to red core disease (P. fragariae) using pot infection technique.

Experiments for comparing the efficiency of different screening techniques for determination of resistance in tomato to P. infestans were conducted in Leningrad (Khrobrikh, 1957). Detached leaves inoculated with the fungus were found to be the most susceptible than cut shoots dipped in zoospore suspension, when 110 varieties of tomato were tested.

Carpenter and Furr (1962) tested the resistance of young seedlings of 515 citrus and related varieties to root rot caused by *P. parasitica* (Dastur) water h. by immersing their root system in aerated water containing *P. parasitica*. Young seedlings with 10 to 20 leaves were preferred for inoculation. The fungus was cultured on a medium prepared from 20 g frozen green beans made into a slurry and 16 g agar per litre of water. The cultures were flooded with water for 12 to 18 hours to stimulate sporangial production and were crushed by hand under water and passed through a coarse colander just before addition to water in the inoculation tank. Seedlings were inoculated by root immersion method in which the entire root system and the collar zone of seedlings were immersed in aerated water. Plants were transferred from the tank to half shaded beds of peat moss vermiculite at 25 to 30°C and kept for atleast two to three months. After incubation, survivors were planted in soil infested with *P. parasitica* for further testing and observation.

Guzman (1965) tried two methods of selection for partial resistance to *P. infestans* on potato in

the green house. In the first method, inoculation of individual leaves with a zoospore suspension was done and those leaves in which sporulation occurred in less than eight hours after inoculation were discarded. In the second method, plants were inoculated uniformly with zoospore suspension using a compressor, left in a humidity chamber over night, and assessed for blight after 5 days. However, there was no difference between the two methods. Howard (1965) reported that spraying zoospore suspension of P. infestans on whole tubers of potato was not sufficiently sensitive to indicate the degree of resistance but helped only to distinguish between the resistant and the highly susceptible ones.

Kovachich (1965) screened breeding lines of potato for foliage and tuber resistance against blight disease caused by P. infestans. Foliage resistance was found out by inoculating detached leaves with a drop of zoospore suspension and incubating at 12°C for 24 hours at high relative humidity followed by a further incubation for 72 hours at 25°C. Resistance was rated by the size of the developing lesion. The resistance of tuber flesh was estimated by multiple point wounding through a standard zoospore suspension followed by 14 days incubation in the laboratory.

Lawrence (1978) tested seven methods for finding out the best evaluation method for assessing the resistance of cocoa cultivars and hybrids to *P. palmivora*. He observed that, for fruiting trees the most reliable method was point inoculation of unwounded attached pods, using a zoospore suspension and recording percentage infection and lesion diameter. The most satisfactory method for hybrid progenies was inoculation of pre-germinated peeled seeds with a drop of zoospore suspension and recording the percentage emergence of healthy seedlings.

Kularatne and Jacob (1980) screened 11 cocoa cultivars against black pod by inoculation with infected pod discs. From the middle portion at the side of each pod, a uniform sized disc of tissues was taken out using a cork borer of 0.5 cm diameter. With the same cork borer, a disc of tissue from diseased pod was taken and inserted into the cavity created by the excavation of tissues on the pod for screening. The inoculated pod was kept hanging inside a wire cage of 12 x 12 x 22 cm size, by using a pin on the pod stalk and tying with a string into the frame of the cage. Then the cage with inoculated

pod was enclosed by a polythene bag containing about 10 ml of water to provide high humidity. The rate of spread of lesion and the time taken to reach 25%, 50%, 75% and 100% infection were recorded based on visual observations.

Naidu et al. (1980) tested the reaction of different varieties of citrus, seedlings against P. nicotianae Breda de Haan. var. parasitica (Dastur) Waterh. in the green house by inoculating their root systems with suspension of zoospores in the aerated culture tanks. The amount of inoculum used was at the rate of one culture flask (100 ml capacity) per five seedlings. Seedlings were supported on glass rod and held for 24 hours in the inoculation tank. Inoculated seedlings were replanted individually in polythene bags filled with steam sterilised soil. Disease rating was determined on the basis of severity of symptoms on the tap and feeder roots.

Pillai (1980) tested the relative susceptibility of rubber clones to abnormal leaf fall. He used the artificial inoculation method for screening planting materials to ascertain their susceptibility to disease by inoculating the petioles on cuttings. RRIM 701 was found to be highly susceptible among the ten clones tested, while G 1 was found to be resistant.

Reddy and Nagarajan (1980) screened 406 collections of N. tabacum and N. rustica and 33 Nicotiana species to P. parasitica var. nicotianae using stem inoculation technique. Seedlings were raised in sterilised soil. Spore and mycelial suspension of six days old virulent culture of Phytophthora grown on oat meal agar was prepared in distilled water by macerating in a waring blender and it was inoculated at the base of the plants uniformly. Inoculated plants were kept in humid chambers. Periodical observations on leaf blight and stem infection were taken. External stem lesion length was taken and reaction was evaluated using the grade 0 to 5 mm as resistant and above 5 mm was recorded as susceptible.

Reaction of 155 cultivars of citrus to Phytophthora disease was tested by Sawant et al. (1980). They used the root inoculation method. Measured quantity of the culture per unit area of the soil was added. The seedlings were reinoculated after a period of six weeks. Periodical observations were recorded on the growth, collar rot, root length and number and length of damaged roots. The susceptibility or resistance was graded into the

following five categories on the basis of damage to feeder and tap roots:

1. No visible symptom after repeat inoculation (R)
2. A few roots observed after repeat inoculation (MR)
3. Damage to some roots with no ill effects on the growth of plants (L)
4. Root system damaged upto 50 per cent (MS)
5. Root system either decayed or missing (HS)

Howard (1941) observed that P. cactorum (Lebert and Cohn) Schroet produced toxin in the filtrate of liquid media, which when injected caused mortality to the maple trees. He also found that this toxic effect could be inactivated by the addition of 0.5% aqueous solution of the dihydrochloride salt of a di-amini-azo-benzene plus a solvent and a penetrant and suggested this as a means of plant disease control.

Wolf (1953) reported that filtrates derived from the cultures of P. parasitica var. nicotianae grown in potato dextrose broth for four weeks or longer induced wilting of detached leaves of tobacco and tomato. Similar results were obtained in a number of other media. The time required for the

appearance of toxin varied with the composition of medium. It was also found that toxin production occurred in diseased plant as well as in cultures of the fungus. Later, Wolf and Wolf (1954) determined the extent of toxin production of P. parasitica var. nicotianae on the basis of the amount of wilting induced on detached leaves of tobacco and tomato. Wilting occurred in dilutions of 1:1, 1:5 and 1:10. This toxin was moderately heat stable, non-volatile and dialyzable.

Forrest and Steib (1961) observed evidence of a toxic substance produced by fungi involved in seed piece rot of sugarcane. Sugarcane seed pieces planted in vermiculite were watered with extracts from Rhizoctonia spp. and other rotting organisms like Phytophthora erythroseptica Pethyb., Phytophthora megasperma Dreschsel. and Glomerella tucumanensis. Seedlings watered with the culture filtrate of P. erythroseptica for four days killed 100 per cent seedlings while less concentrated extracts caused different degrees of wilting. Seidel (1961) studied the nutrient requirement and toxin formation of the fungus P. infestans in different synthetic nutrient solutions. He found

that NH_4 and tartarate were the essential factors in the nutrient solutions for production of toxin and of five sugars tested, sucrose proved to be the best sugar for toxin production. He also reported that boiling the filtrate led to a marked reduction in the toxicity of the culture filtrate.

Savel'eva and Rubin (1963) reported that F. infestans produced a polysaccharide in the liquid culture which is toxic to the plants. Savel'eva and Vasyukova (1966) reported that F. infestans produced two types of toxins. They were polysaccharides isolated from the protein lipid polysaccharide complex and the culture medium and proteins from the mycelium. Acetylized amino saccharides were detected in the polysaccharide from the medium which was more toxic than that from the mycelium. Toxin accumulated by F. megasperma Drechs. var. sojae Hild. in soybean broth after seven days was sufficient to cause wilting of one week old seedlings of cultivar Horosoy-63 soybeans (Paxton, 1972). It was also observed that wilting occurred in plants without roots indicating that toxin effect was not primarily on the root system.

Lee (1973) reported that P. palmivora produced a toxin in liquid cultures and the symptoms produced by the inoculation of toxin were same as that of the pathogen.

Phytotoxicity of water soluble β-1-3-glucans from Phytophthora cinnamomi Rands., P. palmivora and P. megasperma var. sojae was reported by Keen et al. (1975) as revealed by wilting symptoms on Persea indica, soybean, cacao and tomato at 0.01 to 0.5 mg/ml concentration. Kuc and Lisker (1976) reported that toxin from P. infestans regulates the phenolic and terpenoid metabolism in potato. Behnke and Lonnendinker (1977) isolated phytotoxic substances from the culture filtrates of the fungus P. infestans.

Csinos and Hendrix (1977) reported laminar necrosis, growth inhibition and death of tobacco plants caused by toxic extracts of cultures on oats and of mycelium of Phytophthora cryptogea Pethybr. and Laff. grown on glucose-glutamate medium in shake cultures. The severity of growth inhibition and foliar necrosis increased with the duration of exposure of the roots to the extracts and long exposures (60 mts) often resulted in plant death. Csinos and Hendrix (1977) also reported that

P. cryptogea produced exotoxin and endotoxin. Sterilized aqeous extract of the mycelium and culture filtrate of the pathogen when administered on excised tobacco leaves, caused water soaking within 12 hours, laminar collapse within 20 hours and extensive dehydration within 48 hours. They also found that leaves collected for bioassay from the glass house in the afternoon were less sensitive than those collected in the morning or at night. The toxin was equally active at 20°C and 27°C of incubation temperature.

Later, Csinos and Hendrix (1978) studied the effect of culture extracts from 53 isolates of *Pythium* and *Phytophthora* and one of *Achlya* on excised tobacco leaves. They found that toxic extracts were obtained from most isolates of *P. cryptogea*, *P. drechsleri*, *P. erythroseptica* and *P. megasperma* not reported to be parasitic on tobacco. Plich and Rudnicki (1979) reported that filtrates from 14 day old culture of *P. cactorum* an isolate from the soil of a mapple orchard contained a toxin which induced wilting of tomato leaves after 6 to 8 hours and browning of veins and the area around the stem after 15 to 24 hours. They also observed maximum toxin production in a medium containing asparagine

as a nitrogen source at 24 to 26°C in the dark. The toxin was stable when autoclaved at 121°C in strong acid but not strong base. It was non-dialysable, had a relative high molecular weight and was hydrophobic.

Woodward et al. (1980) observed that wilt inducing toxins from Phytophthora spp. contained β glucans and glucanhydrolases.

There is only one report of screening of pepper varieties against Phytophthora disease using toxic metabolite of culture filtrate. Lee (1973) tried this method using five cultivars of P. nigrum, P. colubrinum and Piper sarmentosum. He used eight months old rooted cuttings and the roots were immersed in toxin solution prepared from the filtrates of P. palmivora. According to him, the symptoms of foot rot so produced were comparable to those from fungal infection. P. colubrinum and P. sarmentosum were found to be resistant but the five cultures of P. nigrum were found susceptible.

Savel'eva and Rubin (1963) used the polysaccharide excreted by P. infestans in liquid culture for screening the potato varieties.

Materials and Methods

MATERIALS AND METHODS

1. Location of the experiment

The study was conducted at the College of Horticulture, Vellanikkara.

2. Plant materials used for screening

One year old rooted cuttings of sixteen different open pollinated and hybrid seedlings of pepper and the Panniyur-1 variety of pepper (*Piper nigrum* Lin.) were used for the study. The rooted cuttings were raised in pots and were grown under uniform conditions. The rooted cuttings of pepper used for the experiment were obtained from the Pepper Research Scheme, Vellanikkara. Details of the different types and variety of pepper used for screening are given in Table 1.

3. Isolation and purification of the pathogen

The pathogen causing quick wilt of pepper (*Phytophthora palmivora*) was isolated from infected leaves by standard methods and the pure culture of the pathogen was maintained in oat meal agar (Riker and Riker, 1936).

Table 1. Types and variety of *Piper nigrum* Lin. used for screening against *Phytophthora palmivora*

Sl. no.	Culture nos./variety	Parental combinations
1.	15	Uthiramkotta II (OP)*
2.	21	Uthiramkotta II (OP)*
3.	74	Kuthiravally (OP)*
4.	83	Kuthiravally (OP)*
5.	94	Kuthiravally (OP)*
6.	98	Karimunda (OP)*
7.	115	Arikottanadan (OP)*
8.	120	Arikottanadan (OP)*
9.	122	Arikottanadan (OP)*
10.	155	Karinkotta (OP)*
11.	167	Veluthanamban (OP)*
12.	180	Thaliparamba IV (OP)*
13.	188	Thaliparamba IV (OP)*
14.	191	Thaliparamba IV (OP)*
15.	239	Perumkodi (OP)*
16.	309	Karuvilanchy x Kotta-1
17.	Panniyur-1	Uthiramkotta x Cheriyakaniyakadan

*OP - Open Pollinated

4. *In vitro* production of toxin by pathogen in different liquid media

In vitro production of toxin by the pathogen was tested by growing uniform number of hyphal tips. Discs from the seven day old culture from the fungus were grown in 250 ml flask each containing 100 ml of five different liquid media, namely, Richards + yeast extract broth, Potato dextrose broth, Oat meal broth, Corn meal broth and Synthetic liquid medium for *Phytophthora* (Ainsworth, 1971; Singh, 1975).

The composition of the different media is given below:

Richards + yeast extract broth

Potassium nitrate	..	10 g
Potassium dihydrogen phosphate	..	5 g
Magnesium sulphate	..	2.5 g
Ferric chloride	..	0.02 g
Sucrose	..	50.00 g
Distilled water	..	1,000 ml
Yeast extract	..	2.5 %

Potato dextrose broth

Potato	200 g
Dextrose	20 g
Distilled water	1,000 ml

Oat meal broth

Oats	60 g
Distilled water	1,000 ml

Corn meal broth

Corn meal	60 g
Distilled water	1,000 ml

Synthetic liquid medium for Phytophthora (Singh, 1975)

Glucose	20 g
Asparagine (hydrated)	2.5 g
$Mg\ SO_4 7H_2O$	0.5 g
$K_2H\ PO_4$	1 g
Thiamine	500 µg
Distilled water	1,000 ml

The conical flasks were incubated at 22 ± 1°C in a B.O.D. incubator for 15 days. Thereafter, the cultures were filtered through Whatman No. 40 filter paper. The filtrate was was centrifuged at

1,000 g for 15 minutes and the pellets were removed to make them free from propagules. The supernatent liquid collected was made up to 100 ml and dialysed against glass distilled sterile water for two hours.

A bio-assay was conducted with five different treatments (propagule-free dialysed culture filtrate of different liquid media) and four replications in order to test in vitro production of toxin by the pathogen. The propagule-free dialysed culture filtrate collected from different liquid media were dropped evenly on the surface of five leaves at 0.05 ml per leaf in a pepper plant of the variety Panniyur-1 after gently pricking the area with sterile needle. Equal number of leaves of pepper plant was treated by the same method with sterile dialysed liquid media. This served as the control. Plants under different treatments were covered with a bell jar containing moist cotton to provide high humidity and were observed for development of necrotic spots.

5. Techniques for screening of *P. nicrum* types and variety

Types and variety of *P. nicrum* (sixteen types and one variety mentioned earlier) were inoculated at saturated humidity and at a temperature of 22 ± 4°C and these were screened against the pathogen adopting several techniques, namely, culture disc, zoospore suspension and propagule-free dialysed culture filtrate on different parts of plants such as detached and undetached leaves, stem cuttings and roots of one year old potted plants.

5.1. Culture disc

For this one week old culture of *Phytophthora palmivora* grown in oat meal agar was used.

5.2. Zoospore suspension

Zoospore suspension was prepared from one week old culture of *P. palmivora* grown in oat meal agar (Turner, 1967). Cultures in petrishes of 8.5 cm diameter grown in oat meal agar medium were placed in conical flasks and were covered with Petri's solution to induce sporangial production.

On the fourth day, the solution was replaced by distilled water at the rate of 250 ml per culture and was incubated at 20 ± 1°C in a B.O.D. incubator over night. Afterwards, the suspension containing zoospores of the fungus was filtered through a single layer of sterile muslin cloth.

5.3. Propagule-free dialysed culture filtrate

The hyphal tips of the pathogen grown in Richards + yeast extract medium for 15 days were dialysed and this was assayed as described earlier.

5.4. Leaf inoculation and bioassay of propagule-free dialysed culture filtrate

The mature and physiologically active leaves of sixteen types and variety Panniyur-1 of P. nigrum were inoculated using culture disc and propagule-free dialysed culture filtrate.

5.4.1. Inoculation using P. palmivora culture disc

Detached leaves as well as leaves of potted pepper plants were inoculated with culture disc of 5 mm diameter (containing both mycellia and

sporangia) on the lower surface of the leaves and swabbed with sterile moist cotton wool. Discs were cut from sterilized culture medium and placed as above to serve as control. The inoculated leaves and plants were provided with high humidity and were observed for the production of necrotic lesions. The diameter of the lesions produced was recorded.

5.4.2. Bioassay of propagule-free dialysed culture filtrate of *P. palmivora*

Detached leaves and leaves of potted plants were administered with propagule-free dialysed culture filtrate. The methodology was similar as in the case of bioassay of propagule-free dialysed culture filtrate described earlier. The detached leaves were kept in moist sterile petridishes for the entire period of observation.

5.5 Stem inoculation and bioassay of propagule-free dialysed culture filtrate

Three noded cuttings/runner shoots (without leaves) were inoculated with zoospore suspension and bioassayed with propagule-free dialysed culture filtrate.

From the runner shoots, cuttings were selected from the first half of its length and the remaining tender portions were discarded. Fresh cuttings were made under sterile water and before putting the cuttings in the propegule-free dialysed culture filtrate, or in zoospore suspension, one drop of water was allowed to adhere to the cut end. This was done to avoid the development of air pockets in the conductive tissues. Forty cuttings were used in one replication and these were replicated five times.

5.5.1. Inoculation using *P. palmivora* zoospore suspension

Sets of five cuttings were placed in 250 ml conical flasks containing 50 ml of zoospore suspension and were observed after 24 hours for necrotic lesions by splitting the cuttings longitudinally into two halves and measuring the discoloured area. Cuttings placed in conical flasks containing 50 ml sterile water served as control.

5.5.2. Bioassay of propagule-free dialysed culture filtrate of *P. palmivora*

In the case of propagule-free dialysed culture filtrate, the cuttings were placed in conical flasks containing 50 ml of propagule-free dialysed culture filtrate. The same quantity of dialysed Richards + yeast extract liquid medium was used as control. The methodology and observation were similar to those of zoospore suspension.

5.6. Root inoculation and bioassay of propagule-free dialysed culture filtrate

One year old rooted cuttings of pepper (potted plants) were taken. The roots were washed in running water and in sterile water to remove adhering soil and were inoculated with zoospore suspension and bioassayed with propagule-free dialysed culture filtrate. Twenty five rooted cuttings of each type/variety were used in one replication and these were replicated thrice in each method.

5.6.1. Inoculation using zoospore suspension

In the case of inoculation with zoospore

suspension, the cuttings were placed in 500 ml conical flasks containing 250 ml zoospore suspension. After a period of 24 hours, the inoculum was removed and instead a nutrient solution was kept for a period of six days. The roots were examined daily for lesion development.

The rooted cuttings of Panniyur-1 pepper plant were prepared in the same manner and were placed in sterile nutrient solution. This served as control.

5.6.2. Bioassay of propagule-free dialysed culture filtrate of *P. palmivora*

In the case of propagule-free dialysed culture filtrate, the rooted cuttings of the types and variety were placed in 500 ml conical flasks containing 250 ml of propagule-free dialysed culture filtrate and kept for five days. Instead of propagule-free dialysed culture filtrate, sterile dialysed Richards + yeast extract liquid medium served as control. The methodology adopted and the observations were similar to those of zoospore suspension.

Results

RESULTS

In vitro production of toxin by the pathogen in different liquid media

Five liquid media were tested for the production of toxic metabolite by the pathogen (Table 2).

In all the liquid media tested, the propagule-free dialysed culture filtrate administered on leaves produced necrotic spots which are quite typical for artificial and natural infection of *P. palmivora* (Plate I). However, leaves inoculated with culture media without fungal growth (to serve as control) did not produce the spots.

The maximum symptom expression was observed in Richards + yeast extract medium. After 24 hours of administration of the culture filtrate of this medium, an average necrotic area of 4.2 diameter developed. The culture filtrate of potato dextrose broth gave a mean necrotic area of 2.18 mm diameter. The minimum necrosis was observed in the case of oat meal broth (1.52 mm dia) followed by corn meal broth (1.71 mm dia)(Table 2).

Table 2. Comparison of different media on toxin production by *P. palmivora* (Toxin assayed as average diameter of necrotic spots formed on the leaves of *P. nigrum* variety Panniyur-1 in mm)

Sl. no.	Name of media	Mean symptom development in mm				
		1st day	2nd day	3rd day	4th day	5th day
1.	Richards + yeast extract broth	4.20	10.28	13.19	14.81	14.81
2.	Potato dextrose broth	2.18	6.75	9.25	10.25	10.25
3.	Corn meal broth	1.71	5.28	7.75	8.56	8.56
4.	Oat meal broth	1.52	4.50	7.13	7.63	7.63
5.	Synthetic medium for *Phytophthora*	2.14	6.44	8.69	9.56	9.56
	CD (0.05)					1.654

1 2 5 3 4

PLATE I

Symptom expression on the leaves of *Piper nigrum* (Panniyur-1)

1. Natural infection
2. Inoculation with *P. palmivora* culture disc
3. Assay of propagule-free dialysed culture filtrate of *P. palmivora*

These necrotic lesions further developed very quickly on the second day. It was 10.20 mm diameter for the Richards + yeast extract culture filtrate and 6.75 mm for potato dextrose broth culture filtrate. The least (4.50 mm) was observed in oat meal broth culture filtrate (Table 2).

On the third day also increases in necrotic area were noticed and it was 13.19 mm diameter in the Richards + yeast extract culture filtrate and 7.13 mm for the oat meal broth. All other culture filtrates showed substantial increase in the necrotic area.

There was only slight increase in the necrotic area on the fourth day, after the administration of culture filtrate, when compared to the third day observation. Here also, the maximum diameter of necrotic area on the leaves were observed in the propagule-free dialysed culture filtrate of Richards + yeast extract broth (14.81 mm) followed by potato dextrose broth (10.25 mm). The least was in the case of oat meal broth (7.63 mm) followed by corn meal broth (8.56 mm diameter).

On the fifth day after the administration of culture filtrate, there was no further development in necrotic area as compared to the area in the fourth day (Fig. 1).

Statistical analysis of the fifth day data shows that the maximum necrotic area on leaves are observed in the case of Richards + yeast extract broth. Next to this is the potato dextrose broth followed by thiamine enriched synthetic medium for *Phytophthora* which is on par with the former (Fig. 2).

Techniques for screening *Piper nigrum* types and variety against *P. palmivora*

Inoculation of detached leaves with culture disc

Leaves of sixteen open pollinated and hybrid seedlings and of the Panniyur-1 variety were inoculated with *P. palmivora*. Necrotic spots started appearing from the second day onwards. However, under the high humid conditions, most of the leaves became discoloured and decayed. Due to this, observations could not be taken beyond the fifth day (Table 3). Plants inoculated with culture media without fungal growth did not produce any necrotic spots.

Fig 1 - COMPARISON OF DIFFERENT MEDIA ON TOXIN PRODUCTION BY *Phytophthora palmivora*

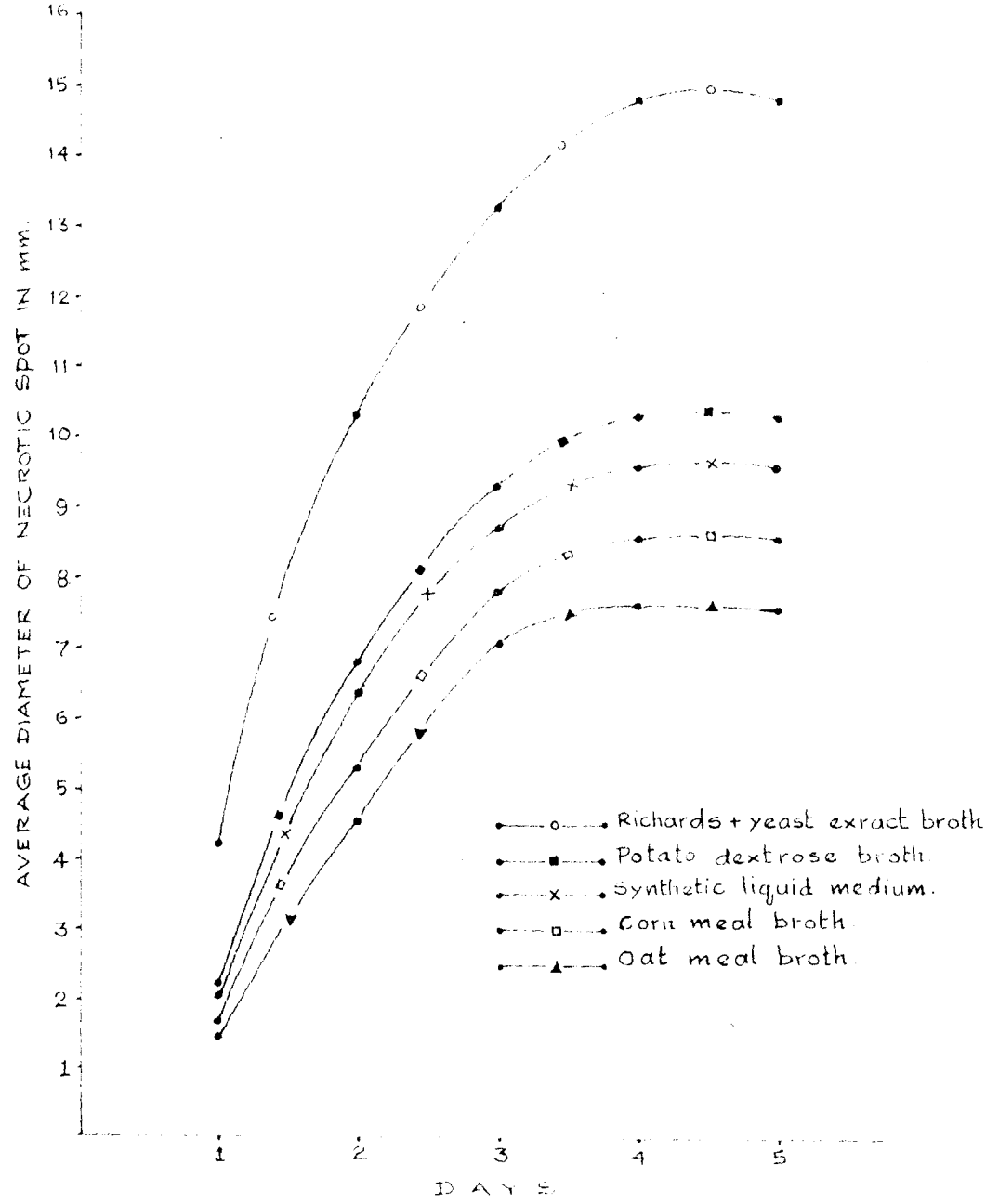


Fig 2 - COMPARISON OF DIFFERENT MEDIA ON TOXIN PRODUCTION BY *Palmivora* ON THE FIFTH DAY.

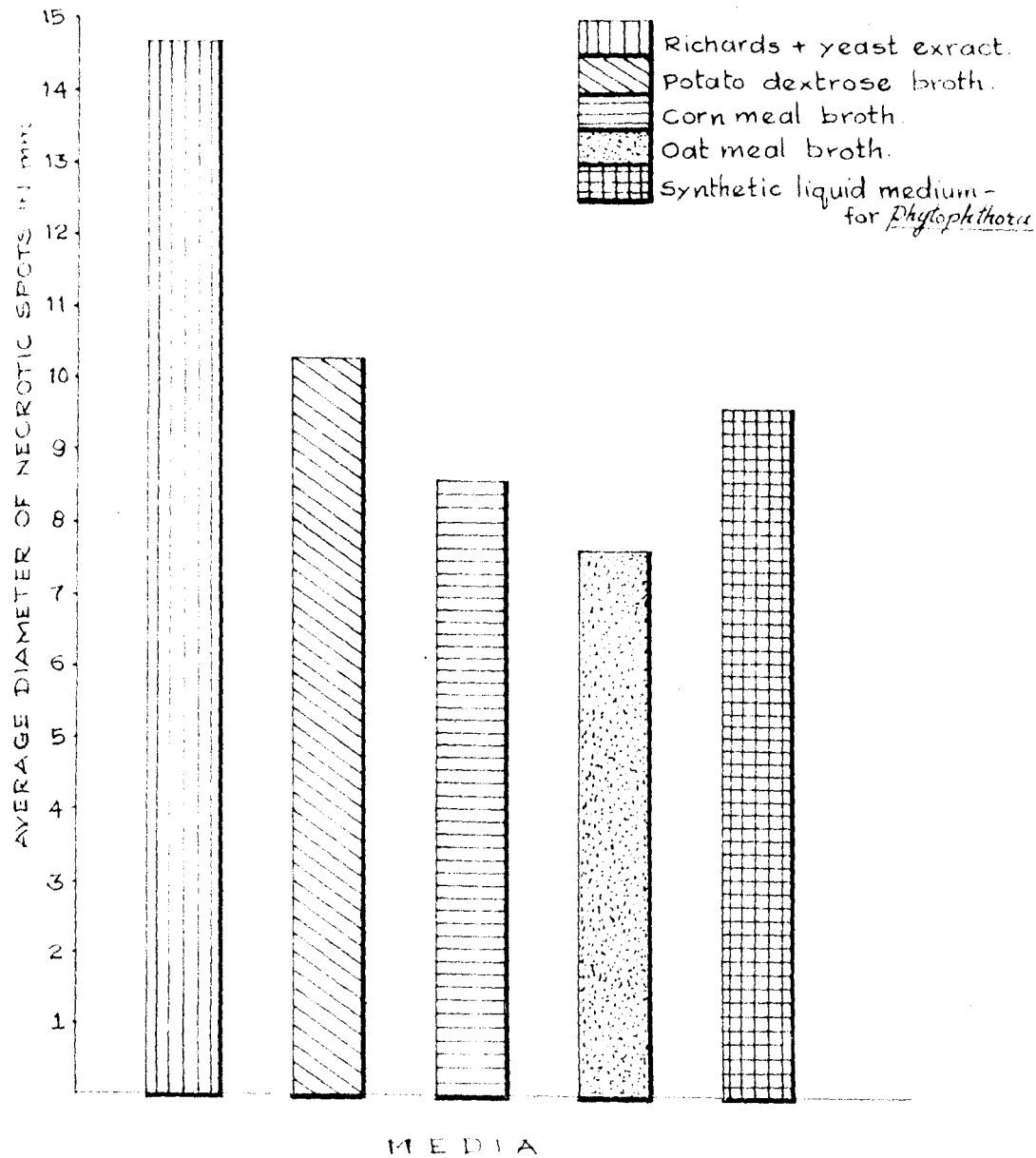


Table 3. Reaction of detached leaves of different types and variety of *P. nigrum* inoculated with culture disc of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean diameter of necrotic spots in mm			
		2nd day	3rd day	4th day	5th day
1.	15	0.73	9.20	24.10	31.63
2.	21	0.43	10.13	25.67	31.87
3.	74	0.93	10.50	24.87	31.07
4.	83	0.67	13.00	23.53	31.67
5.	94	0.80	9.53	24.43	32.17
6.	98	1.17	10.67	26.07	32.80
7.	115	1.80	15.60	28.33	32.40
8.	120	0.73	11.17	26.33	33.00
9.	122	1.13	14.00	24.97	33.00
10.	155	0.80	12.33	25.47	32.13
11.	167	0.53	12.00	23.43	33.00
12.	180	0.77	13.33	24.33	31.83
13.	188	1.20	14.67	25.87	33.00
14.	191	1.47	12.47	27.50	31.63
15.	239	1.17	14.00	27.57	32.80
16.	309	0.77	11.83	24.10	31.33
17.	Panniyur-1	1.93	14.77	28.23	32.97

The minimum average diameter of the necrotic area on the second day was in culture number 21 (0.43 mm) followed by culture number 167 (0.53 mm). The maximum average diameter of the necfotic area (1.93 mm) was observed in the variety Panniyur-1 (Table 3).

On the third day, there was changes in the development of symptom in different cultures. The maximum average diameter of the necrotic area was observed in the culture number 115 (15.60 mm) followed by the hybrid Panniyur-1 (14.77 mm). The P. nigrum culture number 15 (9.20 mm) followed by culture number 94 (9.53 mm) were found to have the least diameter for the necrotic area (Table 3).

The development in the necrotic area was found to be very fast in all the types when compared to the second day and this ranged from 8.47 mm to 14.10 mm diameter. The maximum increase in development of necrotic area was found in culture number 115 (14.10 mm) followed by the culture number 188 (13.47 mm dia), whereas the minimum enlargement in diameter of the necrotic area was in the culture number 15 (8.47 mm) followed by the culture number 94 (8.93 mm).

But on the fourth day, the intensity of the symptom expression showed changes and the minimum necrosis measured in diameter was in the culture number 167 (23.43 mm) followed by the culture number 83 (23.54 mm). The maximum, as that of the third day was 28.33 mm in culture number 115 followed by 28.23 mm in the Panniyur-1 (Table 3).

On the fourth day also, the necrotic area developed very fast as in the case of previous day except in the culture number 94, where further development of necrotic area was only to the extent of 4.90 mm diameter. In all other cases it ranged from 10.53 mm to 15.54 mm diameter. The maximum necrosis was in the culture number 21 and the culture number 83 was found to have the minimum necrotic area.

On the fifth day also, the expression of disease intensity varied among the types, the maximum average diameter of necrotic area being in the culture number 167 (33.00 mm) with the same intensity in the culture numbers 120, 122 and 188. The minimum necrosis in diameter was found in the culture number 74 (31.07 mm) followed by the culture number 309 (31.33 mm) and the culture number 21 (31.57 mm). All the other cultures showed

the intermediate levels of symptom expression.

Observations on the fifth day showed that further development of necrotic area was not the same extent as in the previous day and this ranged from 9.87 to 9.57 mm in diameter. In this case, the maximum was found in the culture number 167 and the minimum was in the Panniyur-1 variety.

In general, it was found that there was no consistency in the development of necrotic area in each of the successive days after the inoculation of P. palmivora on detached leaves of different cultures and the variety Panniyur-1. The statistical analysis of data for the fifth day reveals that there is no significant difference between the cultures and the variety tested with reference to the development of symptoms consequent on inoculation with P. palmivora culture (Table 3).

Bio-assay of detached leaves with propagule-free dialysed culture filtrate

The best medium for the maximum production of toxin was found to be the Richards + yeast extract

broth. Fifteen days old propagule-free dialysed culture filtrate was used for screening the plants.

Sixteen types of P. nigrum and the variety Panniyur-1 were screened with the propagule-free dialysed culture filtrate with the check as described in 'materials and methods' and observations were recorded (Table 4). None of the leaves in the check showed any symptoms even after five days of observation. But in plants which were treated with propagule-free dialysed culture filtrate, the symptoms typical of the natural infection were observed after 24 hours of administration of culture filtrate in all the pepper types/variety tested. The symptom was very conspicuous.

Twenty four hours after the treatment, the mean necrotic area in diameter in the different types of P. nigrum ranged from 12.63 mm to 14.96 mm. The least area was in culture number 309 and culture number 120 showed the maximum necrotic area (Table 4).

On the second day, the lesion development slightly increased in all the types of P. nigrum tested, and the diameter of the necrotic area ranged from an average of 14.90 mm to 17.37 mm (Table 4).

Table 4. Reaction of detached leaves of different types and variety of *P. nigrum* bio-assayed with propagule-free dialysed culture filtrate of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean diameter of necrotic spots in mm				
		1st day	2nd day	3rd day	4th day	5th day
1.	15	13.43	14.90	16.43	17.17	17.17
2.	21	13.63	16.47	17.57	18.03	18.03
3.	74	13.03	16.03	17.23	17.93	17.93
4.	83	13.50	16.73	17.30	17.83	17.83
5.	94	14.77	16.97	17.73	18.27	18.27
6.	98	14.73	17.03	17.83	18.13	18.13
7.	115	14.27	17.13	18.23	18.40	18.40
8.	120	14.97	17.17	18.47	18.87	18.87
9.	122	14.37	17.37	18.73	19.00	19.00
10.	155	14.50	16.43	18.17	18.43	18.43
11.	167	13.60	17.20	18.77	19.00	19.00
12.	180	14.03	15.90	17.23	17.87	17.87
13.	188	13.23	16.63	18.10	18.47	18.47
14.	191	13.87	16.10	17.27	17.83	17.83
15.	239	14.73	16.90	17.97	18.37	18.37
16.	309	12.63	15.40	16.47	17.50	17.50
17.	Panniyur-1	14.83	16.47	18.17	18.57	18.57

Third day after inoculation, further development of the necrotic area was very slow and it ranged from an average diameter of 16.43 mm to 18.77 mm. However, there was not much difference in the expression of symptoms by the different cultures, as compared to second day. Here also, culture number 15 showed the minimum average diameter of the necrotic area (16.43 mm), followed by the culture number 309 (16.47 mm), while the culture number 122 (18.73 mm dia) and the culture number 167 (18.33 mm dia) were found to have the maximum average necrotic areas.

The observations on the further development of necrotic area did not show any appreciable changes, the range in diameter being from 17.17 mm to 19.00 mm, from the third day. The intensity of the symptom in different cultivars was almost of the same grade as observed on the third day (Table 4).

On the fifth day after the administration of the culture filtrate, there was no further development of necrotic area as compared to that in the fourth day.

Observation on the fifth day showed that there was only very little difference in mean necrotic area (1.83 mm dia) of different types and variety of P. nigrum when assayed against the propagule-free dialysed culture filtrate of the pathogen.

The minimum average values were observed in the case of the culture number 15 (17.17 mm) followed by culture number 309 (17.50 mm dia), whereas the culture number 122 and 167 were found to have the maximum average necrotic area (19.00 mm dia)(Table 4).

Statistical analysis of the data revealed that there was no significant differences among the types and variety tested, when propagule-free dialysed culture filtrate of P. palmivora was administered on detached leaves after five days.

Inoculation with culture disc of P. palmivora on leaves of potted pepper vine

As described in 'Materials and Methods', the culture disc of the pathogen were tested on un-detached leaves of different types to study their reaction to the symptom expression.

The necrotic areas on the leaves of different cultivars were observed on the second day after inoculation. There was slight variation in the size of the necrotic area and the mean values ranged from 0.13 mm to 0.28 mm. The maximum average diameter of necrotic area was observed in the culture number 239 (0.28 mm) followed by the culture number 188 (0.27 mm) as against the minimum average diameter of 0.13 mm for the culture number 191 and 0.15 mm for the culture numbers 74 and 155 (Table 5).

On the third day, the diameter of the necrotic area ranged from an average of 3.92 mm to 5.25 mm and all the types showed appreciable changes in the expression of symptoms. A different trend in the symptom development was also noticed among the types as compared to the second day. There was an increase of the necrotic area ranging from 3.74 to 5.00 mm diameter as compared to the previous day. The least necrotic area was in the culture number 309 with an average diameter of 3.92 mm followed by the culture number 94 (4.25 mm) and the maximum values were 5.25 mm and 5.16 mm for hybrid Panniyur-1 and the culture number 21, respectively (Table 5).

Table 5. Reaction of undetached leaves of different types and variety of *P. nigrum* inoculated with culture disc of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean diameter of necrotic spots in mm			
		2nd day	3rd day	4th day	5th day
1.	15	0.22	4.85	12.71	18.63
2.	21	0.23	5.16	11.91	19.47
3.	74	0.15	4.75	11.08	17.88
4.	83	0.22	4.75	11.33	19.25
5.	94	0.18	4.25	10.95	18.55
6.	98	0.18	4.83	11.75	18.67
7.	115	0.22	4.92	11.13	20.21
8.	120	0.22	4.75	11.41	20.41
9.	122	0.18	4.50	11.20	20.45
10.	155	0.15	4.70	11.08	20.17
11.	167	0.25	4.78	11.75	19.67
12.	180	0.20	4.58	10.93	19.50
13.	188	0.27	4.75	11.58	20.41
14.	191	0.13	4.58	11.05	18.67
15.	239	0.28	4.75	11.68	21.30
16.	309	0.18	3.92	10.66	19.41
17.	Panniyur-1	0.25	5.25	11.58	21.50
	CD (0.05)				1.60

On the fourth day after inoculation, the necrotic area furth r increased rapidly in all the types and the extent of increase in diameter was 6.21 mm to 7.86 mm. The average diameter of the necrotic area of different types of pepper ranged from 10.66 mm to 12.71 mm. There was slight variation in the intensity of symptom expressed in different types as compared to that of the third day, but the minimum was observed in the culture number 309 as in the case of third day. The culture number 15 showed maximum necrosis.

Further development of symptom was observed in all the plants tested on the fifth day. The average diameter of the necrotic area of different types varied from 17.88 mm to 21.50 mm. But the maximum necrotic area was detected in the hybrid Panniyur-1 (21.50 mm dia), followed by the culture number 239 (21.30 mm). The culture numbers 74 (17.88 mm), 94 (18.55 mm) and 15 (18.63 mm) were found to have the minimum average diameter of the necrotic area (Table 5).

After four days, the development of the necrotic area was appreciably increased within 24 hours and

the increase ranged from an average diameter of 5.92 mm to 10.22 mm. On the sixth day it was observed that most of the leaves inoculated got detached from the vines and further observations could not be recorded (Table 5).

Statistical analysis of the data for the fifth day shows that the reaction of undetached leaves of different types and variety of P. nigrum against P. palmivora differs significantly. The minimum development of necrotic area was observed in the culture number 74 which was found to be on par with the cultures, 94, 15, 98, 191, 83, 309 and 21. The maximum development of leaf lesion was found in the variety Panniyur-1 and the culture numbers 239, 122, 180, 188, 115 and 155 were found to be on par (Table 5).

Bio-assay of undetached leaves with propagule-free dialysed culture filtrate

All the pepper types and variety of P. nigrum which were inoculated with culture disc were taken for the study. The propagule-free dialysed culture filtrate was bio-assayed against the undetached leaves and observations were recorded as described

in 'Materials and Methods' (Table 6).

The average diameter of the necrotic areas in different types of P. nigrum varied from 3.92 mm to 4.66 mm, 24 hours after administration with the culture filtrate. The minimum was found in the culture number 309 and the maximum was in Panniyur-1 (Table 6).

On the second day, the necrotic area of all the leaves increased considerably and the average diameter of the necrotic area of different types was found to be between 10.33 mm to 11.33 mm. The minimum was observed in the case of the culture number 160. Culture number 83 showed the maximum lesion development when compared to first day. There was much variation in the mean diameter of the necrotic area among the different types tested (Table 6).

Three days after the administration of the culture filtrate, the necrotic area on the leaves of different pepper vines were not found to increase to the levels as in the second day. Here, the average diameter of the necrotic area ranged from 13.50 mm to 14.41 mm and the minimum was

Table 6. Reaction of undetached leaves of different types and variety of *P. nigrum* bio-assayed with propagule-free dialysed culture filtrate of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean diameter of necrotic spots in mm				
		1st day	2nd day	3rd day	4th day	5th day
1.	15	3.95	11.10	13.83	15.17	15.17
2.	21	4.25	11.25	14.41	15.25	15.25
3.	74	4.16	11.12	13.50	14.75	14.75
4.	83	4.33	11.23	13.91	15.00	15.00
5.	94	4.08	11.20	14.16	15.42	15.42
6.	98	4.50	11.00	14.00	15.42	15.42
7.	115	4.16	10.83	13.75	14.75	14.75
8.	120	4.33	11.00	14.33	15.92	15.92
9.	122	4.33	10.67	13.75	15.17	15.17
10.	155	4.25	10.42	14.41	15.58	15.58
11.	167	4.20	10.91	14.33	15.33	15.33
12.	180	4.00	10.33	13.83	15.00	15.00
13.	188	4.00	11.25	14.00	15.08	15.08
14.	191	4.16	10.83	13.91	14.92	14.92
15.	239	4.60	10.91	14.33	15.67	15.67
16.	309	3.92	11.17	14.00	15.00	15.00
17.	Panniyur-1	4.66	10.67	14.08	15.17	15.17

found in culture number 74 (13.50 mm) while, the culture number 155 recorded the maximum average necrotic area of 14.41 mm diameter (Table 6).

Four days after the administration of culture filtrate on the leaves of different types of pepper, there was no appreciable increase in the necrotic area and the minimum average diameter was 14.75 mm and the maximum, 15.92 mm. Among the pepper types, there was no appreciable differences in the symptom expression after four days. The culture number 74, still showed the minimum, while the culture number 120 was found to have the maximum average necrotic area (Table 6).

On the fifth day after administration of propagule-free dialysed culture filtrate, there was no difference in the necrotic area development in different pepper types and the trend was static.

The statistical analysis of the data reveals that there is no significant difference among the types when the propagule-free dialysed culture filtrate was administered on leaves of potted pepper vines.

The inoculation of pathogen and administration of propagule-free dialysed culture filtrate on the detached leaves and leaves of potted pepper plants, five days after the treatment, clearly showed that there was a uniformity in the symptom expression in all the types and variety tested (Table 7 and Fig. 3). The maximum symptom development was observed on detached leaves inoculated by the culture disc. It ranged from an average diameter of 31.07 mm to 33.00 mm. On the leaves of potted pepper vine, the minimum average symptom expression was 17.88 mm as against the maximum of 21.50 mm. But on detached leaves, the pathogen was found to grow very well causing larger necrotic area (Table 7 and Fig. 3).

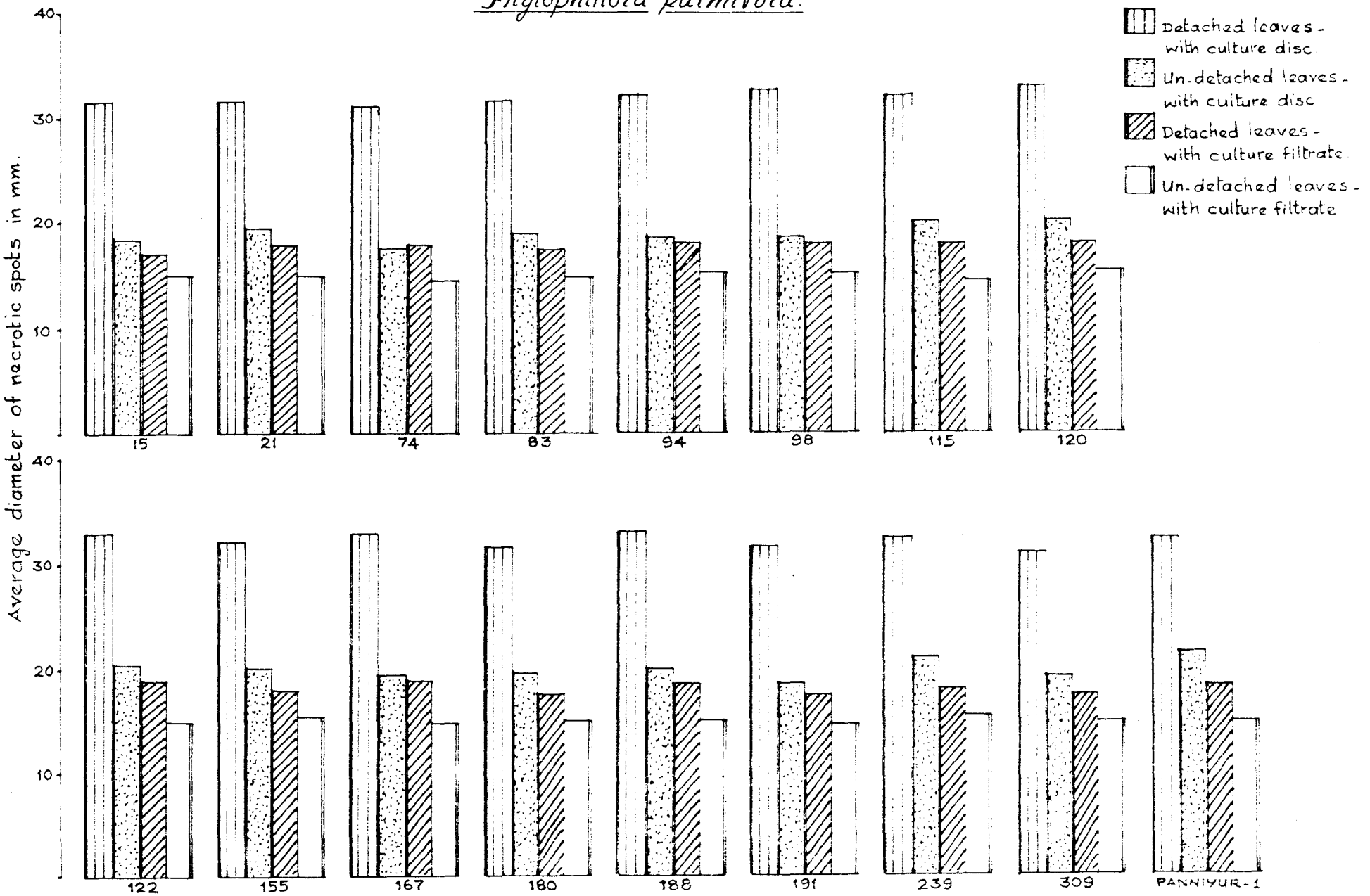
When the propagule-free dialysed culture filtrate was administered, there was not much difference in symptom expression in the detached leaves and leaves of potted pepper vine, even though, the detached leaves had slight increase in symptom expression as compared to that of leaves on potted pepper vine (Table 7).

The results clearly reveal that the Piper types/variety which showed maximum symptom when

Table 7. Reaction of different types and variety of *P. nigrum* after five days of inoculation with culture disc and propagule-free dialysed culture filtrate

Sl. no.	*P. nigrum* Culture nos./variety	Mean diameter of necrotic spots in mm			
		Detached leaves		Undetached leaves	
		Culture disc	Culture filtrate	Culture disc	Culture filtrate
1.	15	31.63	17.17	18.63	15.17
2.	21	31.57	18.03	19.47	15.25
3.	74	31.07	17.93	17.88	14.75
4.	83	31.67	17.83	19.25	15.00
5.	94	32.17	18.27	18.55	15.42
6.	98	32.50	18.13	18.67	15.42
7.	115	32.40	18.40	20.21	14.75
8.	120	33.00	18.87	20.41	15.92
9.	122	33.00	19.00	20.45	15.17
10.	155	32.13	18.43	20.17	15.58
11.	167	33.00	19.00	19.67	15.33
12.	180	31.83	17.87	19.50	15.00
13.	188	33.00	18.47	20.41	15.08
14.	191	31.63	17.83	18.67	14.92
15.	239	32.50	18.37	21.30	15.67
16.	309	31.33	17.50	19.41	15.00
17.	Panniyur-1	32.97	18.57	21.50	15.17
	CD (0.05)	NS	NS	1.60	NS

Fig. 3 - Reaction of leaves of different types/variety of *Piper nigrum* after five days of inoculation with culture disc and propagule-free dialysed culture filtrate of *Phytophthora palmivora*.



inoculated with culture disc on detached and undetached leaves also showed more or less same trend in symptom expression when the propagule-free dialysed culture filtrate of the pathogen was administered.

The minimum average diameter of necrotic area was in culture number 74 (31.07 mm), when the detached leaves were inoculated with culture disc. On potted pepper plant leaves, it was 17.88 mm diameter. In the case of culture filtrate administered on leaves of potted pepper plants also, the same pepper type showed the minimum diameter of 14.75 mm necrotic area (Table 7 and Fig. 3).

Similarly, the maximum symptom expression was observed in culture numbers 120, 122, 188 and 167 (33.00 mm dia) followed by the hybrid Panniyur-1 (32.97 mm dia) and the culture number 239 (32.50 mm dia) when inoculated with living pathogen an detached leaves.

Leaves of potted pepper vine also showed similar necrotic symptom when inoculated with the living pathogen. Almost the same trend was observed when the culture filtrate was administered

on the leaves of pepper types (both detached and undetached) (Table 7 and Fig. 3).

This clearly indicates that inoculation with living pathogen and administration of propagule-free dialysed culture filtrate will serve the purpose of testing the reaction of pepper leaves against the pathogen, *P. palmivora*.

Inoculation of stem cuttings with zoospore suspension

From the different cultures of *Piper nigrum*, three noded fresh cuttings were placed in zoospore suspension and observations were recorded as described in 'Materials and Methods'. The results of these studies are presented in Table 8.

The length of the discoloured area was measured on the second day after placing in zoospore suspension and it ranged from an average length of 7.20 mm to 8.14 mm. The maximum average length of discoloured area was found in the hybrid Panniyur-1 (8.14 mm) followed by the culture number 309 (8.10 mm) and the minimum was in the culture number 94 (7.20 mm) followed by the culture numbers 98 and 188 (7.30 mm). But there was not much difference among the types tested (Table 8).

Table 8. Reaction of stem cuttings of different types and variety of *P. nigrum* inoculated with zoospore suspension of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean length of lesion development in mm			
		2nd day	3rd day	4th day	5th day
1.	15	7.35	13.30	23.80	48.00
2.	21	7.84	14.30	25.30	49.00
3.	74	7.90	14.50	25.50	49.30
4.	83	7.90	13.50	23.20	48.00
5.	94	7.20	12.70	23.70	47.40
6.	98	7.30	13.50	23.60	47.80
7.	115	7.90	14.30	25.40	48.20
8.	120	7.60	13.80	25.30	48.80
9.	122	7.80	13.70	25.00	48.20
10.	155	7.90	13.90	24.80	48.50
11.	167	7.50	12.70	23.30	48.30
12.	180	7.60	13.40	23.90	48.10
13.	188	7.30	12.90	23.70	47.20
14.	191	7.80	14.20	25.00	49.50
15.	239	7.70	13.80	25.10	49.00
16.	309	8.10	14.80	26.00	49.30
17.	Panniyur-1	8.14	14.50	25.30	49.60

On the third day, the lesions developed further and the length ranged from an average of 12.70 mm to 14.80 mm. The minimum average length of the lesion was observed in the culture numbers 94 and 167 (12.70 mm) and the maximum average values were observed in the case of the culture number 309 (14.80 mm) followed by hybrid Panniyur-1 and culture number 74 (14.50 mm). In this case also, appreciable difference could be noticed in the lesion development of different types tested (Table 8).

On the fourth day, the lesion development was very fast as compared to third day and it ranged from an average of 9.70 to 11.50 mm in length. The minimum lesion development on fourth day was 23.20 mm and the maximum was 26.00 mm in length.

The ranking of the cultivars varied from the rating observed in the third day and the minimum average lesion length was observed in the culture number 83. The culture number 309 (26.00 mm) followed by the culture number 74 (25.50 mm) were found to have the maximum average lesion development (Table 8).

As in the case of leaf inoculation, the final observation on lesion development was taken on the fifth day. By that time, the lesion development was very quick as compared to the fourth day and the increase ranged from an average length of 22.80 mm to 25.00 mm. The ranking of types also differed and the minimum average lesion development was observed in the culture number 188 (47.20 mm) followed by the culture number 94 (47.40 mm). The hybrid Panniyur-1 (49.60 mm) followed by the culture number 191 (49.50 mm) were found to have the maximum average lesion length.

Statistical analysis of the data for the fifth day revealed that there was no significant differences among the types and variety when stem cuttings were kept in zoospore suspension (Table 8).

Bio-assay of stem cuttings with propagule-free dialysed culture filtrate

The stem cuttings of the types, which were tested previously for leaf reaction were placed in propagule-free dialysed culture filtrate and

observations were recorded as described in the 'Materials and Methods'. None of the check plants took infection. The observations were taken up to five days and the data are presented in Table 9.

The lesion development observed after the second day ranged from an average length of 26.20 mm to 27.00 mm. The maximum lesion development was observed in the culture numbers 167 and 98 (27.00 mm) and the culture number 309 (26.20 mm) followed by the culture number 94 (26.30 mm) were found to have the minimum average lesion development (Table 9).

On the third day, the lesion development increased further and the range was from 75.20 mm to 76.00 mm. At this stage there was slight variation in symptom expression among the different types, as compared to the position in the second day. The maximum average length of lesion was observed in culture numbers 122 and 98 (76.00 mm) and the minimum was in the culture numbers 94 and 309 (75.20 mm and 75.30 mm. respectively).

The observation taken on the fourth day showed that there was further increase of lesion length in all the types tested. The minimum average length of lesion was observed in culture number 94 (95.30 mm)

Table 9. Reaction of stem cuttings of different types and variety of *P. nigrum* bio-assayed with propagule-free dialysed culture filtrate of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean length of lesion development in mm			
		2nd day	3rd day	4th day	5th day
1.	15	26.56	75.70	96.00	106.80
2.	21	26.90	75.80	96.50	106.10
3.	74	26.80	75.90	96.20	106.00
4.	83	26.30	75.50	96.10	106.60
5.	94	26.30	75.20	95.30	106.20
6.	98	27.00	76.00	96.10	105.70
7.	115	26.40	75.70	96.70	106.20
8.	120	26.90	75.70	96.20	106.10
9.	122	26.90	76.00	96.00	106.30
10.	155	26.60	75.70	96.20	106.90
11.	167	27.00	75.50	95.80	106.50
12.	180	26.80	75.70	96.30	106.70
13.	188	26.60	75.60	95.70	106.10
14.	191	26.60	75.90	96.70	106.70
15.	239	26.70	75.90	96.60	106.80
16.	309	26.20	75.30	96.20	105.90
17.	Panniyur-1	26.50	75.60	96.10	106.70

followed by culture numbers 188 (95.70 mm) and 167 (95.80 mm) and the maximum was found in culture numbers 115 and 191 (96.70 mm) followed by culture number 239 (96.60 mm)(Table 9).

On the fifth day, the development of lesion was very small as compared to the position in the fourth day and this varied from 105.70 mm to 106.90 mm. The maximum average length of lesion was observed in the culture number 155 (106.90 mm) followed by the culture number 180, the hybrid Kanniyan-1 and the culture numbers 191 (106.70 mm) and 83 (106.60 mm). The minimum average length of lesion was observed in the case of culture number 98 (105.70 mm).

Statistical analysis of the data showed that there was no significant difference among the types and the variety in respect of symptom expression when stem cuttings were placed in propagule-free dialysed culture filtrate (Table 9).

On the fifth day, the lesion development ranged from an average length of 105.70 mm to 106.90 mm among the types placed in culture filtrate whereas in zoospore suspension, the length of lesion development was only from 47.20 mm to 49.60 mm.

There was not much difference among the types in length of the lesion whether it was placed in the zoospore suspension or in propagule-free dialysed culture filtrate (Table 10 and Fig. 4). All the pepper types tested were found to be highly susceptible.

Inoculation of roots with zoospore suspension

The rooted cuttings of different types of P. nigrum were inoculated with the zoospore suspension and observations were recorded as described in 'Materials and Methods'. All the types tested were found to be susceptible to the pathogen and these took infection and incited the symptoms. No symptom was detected in control. From second day onwards, the necrotic symptoms were observed on the roots and the observations were taken up to five days. On the sixth day, the entire roots were either decayed or discoloured. The observations recorded on the development of discolouration are presented in Table 11.

On the second day, after inoculation of the roots with zoospore suspension, necrotic lesion development was observed and the measurement of average

Table 10. Reaction of stem cuttings of different types and variety of *P. nigrum* after five days of inoculation with zoospore suspension and propagule-free dialysed culture filtrate of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean length of lesion development in mm	
		Zoospore suspension	Culture filtrate
1.	15	48.00	106.50
2.	21	49.00	106.10
3.	74	49.30	106.00
4.	83	48.00	106.60
5.	94	47.40	106.20
6.	98	47.80	105.70
7.	115	48.20	106.20
8.	120	48.80	106.10
9.	122	48.20	106.30
10.	155	48.50	106.90
11.	167	48.30	106.50
12.	180	48.10	106.70
13.	188	47.20	106.10
14.	191	49.50	106.70
15.	239	49.00	106.50
16.	309	49.30	105.90
17.	Panniyur-1	49.60	106.70

Fig. 4 - Reaction of stem cuttings of different types/variety of *Piper nigrum* after five days of inoculation with zoospore suspension and propagule-free dialysed culture filtrate of *Phytophthora palmivora*.

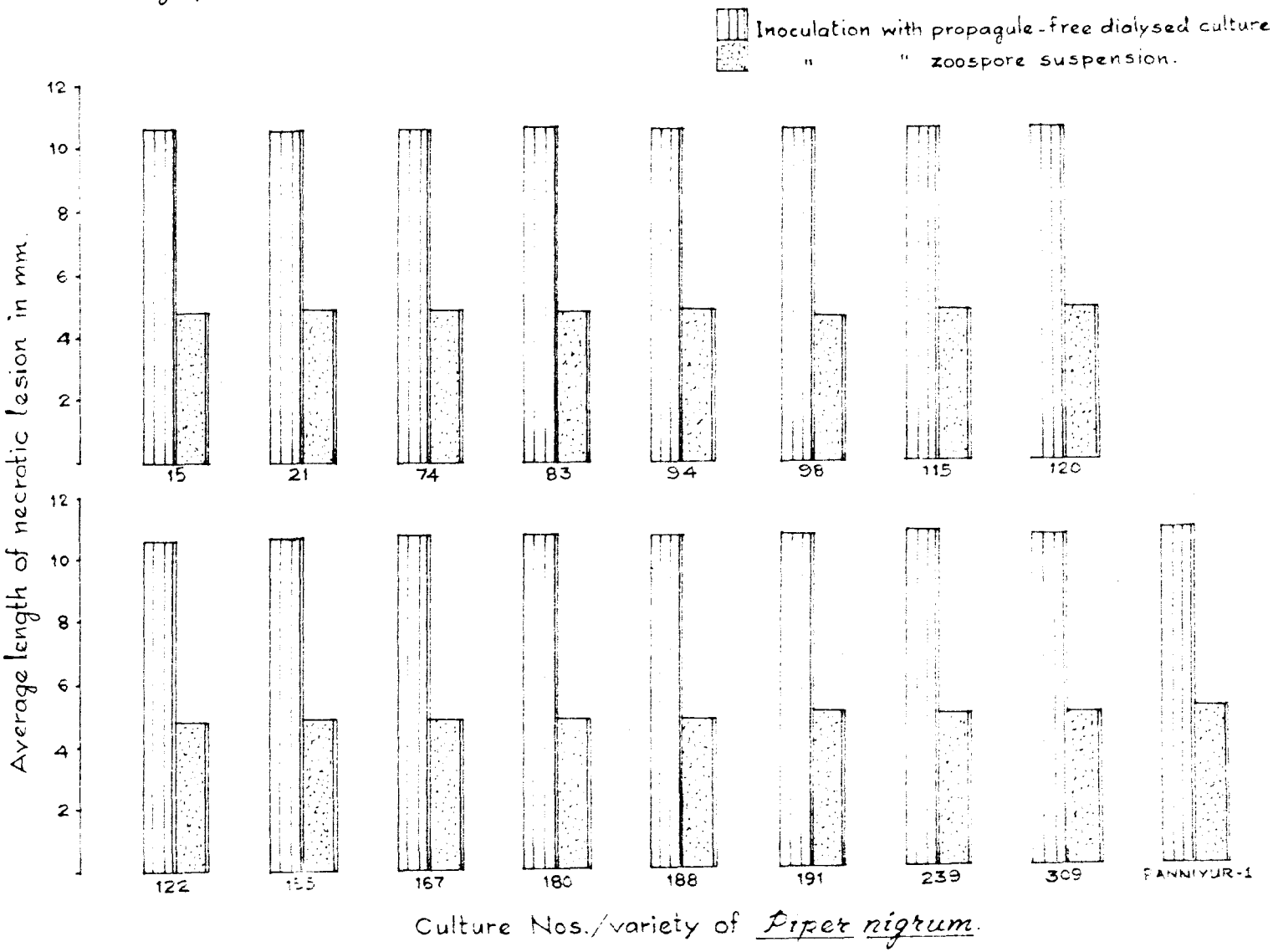


Table 11. Reaction of roots of rooted cuttings of different types and variety of *P. nigrum* inoculated with zoospore suspension of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean length of lesion development in mm				
		2nd day	3rd day	4th day	5th day	6th day
1.	15	26.83	39.50	68.40	93.83	105.66
2.	21	27.03	45.50	71.66	94.50	110.50
3.	74	27.13	41.16	69.33	94.70	107.50
4.	83	26.66	38.50	71.16	94.66	106.66
5.	94	24.30	38.00	65.33	92.53	106.50
6.	98	26.83	40.16	69.66	94.83	108.16
7.	115	26.50	41.33	69.50	95.00	105.50
8.	120	26.83	45.53	73.98	94.33	108.16
9.	122	26.73	40.83	69.83	92.16	107.86
10.	155	26.03	42.16	70.00	94.50	105.66
11.	167	27.46	39.16	70.00	92.66	108.83
12.	180	26.00	45.66	70.50	94.36	106.50
13.	188	27.00	40.86	73.00	93.33	104.50
14.	191	26.40	41.66	68.83	95.16	108.66
15.	239	27.66	42.83	69.66	93.66	106.60
16.	309	26.60	42.23	73.70	95.50	108.16
17.	Panniyur-1	27.66	43.56	74.66	96.00	110.50

length of lesion development ranged from 24.30 mm to 27.66 mm. The maximum lesion development was observed in the culture number 239 and the hybrid Panniyur-1 (27.66 mm). The culture number 94 (24.30 mm) and 180 (26.00 mm) were found to have the minimum average length of lesion (Table 11).

On the third day, further development of discolouration of roots was observed and it ranged from an average length of 38.00 mm to 45.66 mm. In all the types, there was increase in the symptom development but the minimum average length of lesion was observed in the culture number 94 (38.00 mm) followed by the culture number 83 (38.50 mm) and the culture number 167 (39.16 mm). The maximum lesion length was observed in the culture number 180 (45.66 mm) followed by 120 (45.53 mm) and 21 (45.50 mm). When compared to the second day, the increase in lesion development on the roots was much faster on the third day and it ranged from 11.70 mm to 19.66 mm in length (Table 11).

Further progress in symptom expression was noticed on the fourth day, as compared to the expression on the third day and the increase in lesion development ranged from 24.84 mm to 32.66 mm.

The maximum development of lesion on the fourth day was found in hybrid Panniyur-1 (74.66 mm) followed by the culture number 120 (73.90 mm) and 309 (73.70 mm). The culture numbers 94 (65.33 mm), 15 (68.40 mm) and 191 (68.83 mm) were found to have the minimum lesion development (Table 11).

The lesion development on the roots increased further on the fifth day in all the types and it varied from 20.33 mm to 27.20 mm. The minimum average lesion length observed on the fifth day was in the culture number 122 (92.16 mm) followed by the culture numbers 94 (92.53 mm) and 167 (92.66 mm). The hybrid Panniyur-1 (96.00 mm) exhibited the maximum average lesion development followed by the culture number 309 (95.50 mm) and 191 (95.16 mm) in length. No marked difference in the lesion development could be noticed among the types tested.

Observations taken on the sixth day show that irrespective of the length of involved roots, the entire root system was either decayed or discoloured. Measurements of the lesion development were recorded in terms of the average length of the longest roots and the data are presented in Table 11.

Statistical analysis of the data was not possible since the entire roots were decayed within six days in all the P. nigrum types and variety when inoculated with zoospore suspension. In the light of the above facts, the observation recorded on fifth day after inoculation of the roots with zoospore suspension was subject to statistical analysis and the differences were found not to be significant between different types and variety tested.

Bio-assay of roots with propagule-free dialysed culture filtrate

The rooted cuttings of all the P. nigrum types used in the present study were placed in the culture filtrate and the observations were recorded as described in 'Materials and Methods'. Data are presented in Table 12.

All the types tested developed similar symptoms as noticed when inoculated with zoospore suspension. The lesion development was recorded from the second day onwards but the entire roots were found to be either decayed or discoloured after four days.

Table 12. Reaction of rooted cuttings of different types and variety of *P. nigrum* bio-assayed with propagule-free dialysed culture filtrate of *P. palmivora*

Sl. no.	*P. nigrum* Culture nos./variety	Mean length of lesion development in mm		
		2nd day	3rd day	4th day
1.	15	47.50	96.60	109.66
2.	21	49.50	100.50	110.33
3.	74	48.70	95.56	106.60
4.	83	48.33	98.00	107.50
5.	94	48.00	92.83	106.16
6.	98	46.80	97.16	108.33
7.	115	47.70	99.43	108.60
8.	120	49.70	99.80	109.33
9.	122	49.20	99.33	108.00
10.	155	50.23	100.83	108.10
11.	167	47.66	99.66	109.66
12.	180	47.53	100.00	108.50
13.	188	47.66	97.66	108.83
14.	191	49.00	99.86	108.00
15.	239	49.70	98.83	107.66
16.	309	49.50	99.83	110.16
17.	Panniyur-1	50.20	99.33	110.00

When the rooted cuttings were placed in propagule-free dialysed culture filtrate, the lesion development on the second day was very fast and it ranged from an average length of 46.80 mm to 50.23 mm. The maximum average lesion development was in the culture number 155 (50.23 mm) followed by the Panniyur-1 variety (50.20 mm) while the culture numbers 98 (46.80 mm), 15 (47.50 mm) and 180 (47.53 mm) were found to have the minimum lesion development.

On the third day also, the lesion development was very fast and this ranged from an average length of 92.83 mm to 100.83 mm in different types. The minimum average length of lesion development was in the culture number 94 (92.83 mm) followed by the culture numbers 74 (95.56 mm) and 98 (97.16 mm). The culture numbers 155 (100.83 mm), 21 (100.50 mm) and 188 (100.00 mm) were found to have the maximum lesion length (Table 12).

On the fourth day after placing the roots in the culture filtrate, the entire root length of all types was found either rotten or discoloured. The variation in the measurement of the different types was only due to the variation in the length of the longest root. As the entire root length of all types was damaged, the data could not be subjected to statistical analysis. Further observations were not recorded.

Discussion

DISCUSSION

The quick wilt (Foot rot) disease of pepper caused by *Phytophthora palmivora* is a destructive disease of *Piper nigrum* all over the pepper growing tracts of the world. Integrated disease management, with stress on phyto-sanitation, cultural practises and chemical control has been the strategy followed in the past to control this important disease (Muller, 1936; Holliday and Mowat, 1963; Nambiar and Sharma, 1976; Anonymous, 1976; 1978; 1981 and Mammootty *et al.* 1980). However, during epiphytotics, rapid control cannot be expected from the above strategy of integrated management. In such situations, the problem can be tackled effectively by putting resistant/tolerant varieties under cultivation.

P. nigrum is highly heterogenous in nature. It is a matter of common observation that seedlings grown from the seeds of one variety show marked differences and none of them will be true to the parental type. It is well known that the centre of origin of *P. nigrum* is the even green moist forests of Western Ghats and diversity of *Piper* species

is noticed in the submountainous region of these tracts (Gamble, 1918; Purseglove, 1968). Further, over 200 cultivars of pepper are being cultivated in Kerala and they are reported to be highly variable and heterozygous (George and Mercy, 1978). Exploitation of natural heterogenicity of the crop and diversified characters of newly evolved open pollinated and hybrid seedlings can be expected to be quite valuable to develop varieties resistant to this very serious disease.

Information on the resistance of black pepper and allied species is meagre (Muller, 1936; Holliday and Mowat, 1963; Ruppel and Almeyda, 1965; Leather, 1967; Turner, 1971; 1973; Alconero et al., 1972; Sarma and Nambiar, 1979; Sarma et al., 1980). The reaction of large collections of wild types of Piper spp. and large number of open pollinated and hybrid seedlings of cultivars to infection by the pathogen can be studied successfully only if a rapid screening technique is available.

Many pathogenic fungi and bacteria are known to produce toxins, which are injurious to the hosts and some of such substances are disease determinants.

These toxic metabolites produced by the pathogens, play a significant role in pathogenesis.

For the last few decades, much attention was focussed on the role of toxin in pathogenic plant diseases. A good number of plant pathogenic fungi which cause destructive plant diseases has been studied in detail. This include Helminthosporium victoriae causing the victoria blight of oats (Meehan and Murphy, 1946; 1947; Wheeler and Luke, 1954), Alternaria kikuchiana, the casual agent of black spot of Japanese pear (Tanaka, 1933; Hiroe and Aoe, 1954; Hiroe et al. 1958), Periconia circinata causing the milo disease of sorghum (Leukel and Pollak, 1947; Leukel, 1948), Piricularia oryzae, the casual organism of blast of rice (Tamari and Kaji, 1954; 1955), Ceratocystis fimbriata causing sweet potato rot (Uritani and Akazawa, 1959), Colletotrichum gleosporioides causing a number of leaf spot and leaf blight diseases (Nair and Ramakrishnan, 1973), and Phytophthora infestans causing late blight of potato (Bonnebeck, 1956; Savel'eve and Rubin, 1963; Seidel, 1961). Similarly a large number of plant pathogenic bacteria like Pseudomonas tabaci, the casual agent of wild fire of tobacco

(Clayton, 1933; Braun, 1959). *Pseudomonas morsprunorum* causing the bacterial canker and leaf spot of many fruit trees (Erikson and Montgomeri, 1945) and *Pseudomonas phaseolicola* causing the halo blight of beans (Muller, 1950; Waits and Schwarz, 1956) were found to produce powerful toxins both in culture and in host.

Yoder (1981) stated that meaningful studies of the role of pathogen produced toxins in diseases depend on assays. Further, a toxin is originally defined by bioassay.

In the present study, the propagule-free dialysed culture filtrates of *P. palmivora* in different liquid cultures were assayed against *P. nigrum* leaves. The symptom expression was almost the same as in the case of natural infection by the pathogen (Plate I). The criteria prescribed for a phytotoxin by Graniti (1972) has been fulfilled by the above tests. From the present study, it can be reasonably presumed that the propagule-free dialysed culture filtrate of *P. palmivora* in different media having toxic metabolite is injurious to the host, *P. nigrum*. This finding is in conformity with the results obtained by Lee (1973).

The production of toxins by different organisms varies widely according to the culture conditions, nutrients, nutrient sources, pH of the medium etc. (Shaw, 1981). In the present studies, out of the five media tested, maximum toxin production were obtained in Richards + yeast extract liquid medium (Table 2). The ability of plant pathogenic micro-organisms to produce relatively more of toxic metabolites in a complex medium is already established by many workers (Demain, 1968; Hoitink and Sinden, 1970; Drew and Demain, 1977; Gilchrist and Grogan, 1977; Mitchell, 1978). In a synthetic medium when yeast extract is added, this becomes a complex media. The ability of plant pathogenic micro organisms to produce more toxic metabolites in synthetic media enriched with yeast extract has been reported also by Gilchrist and Grogan (1977). The present study confirmed these earlier findings.

In the present investigation, types and varieties of *F. nigrum* were screened against the quick wilt pathogen employing viable inocula as well as propagule-free dialysed culture filtrate containing toxic metabolite of the pathogen.

In both the methods, symptom developed on different plant parts were similar indicating the possibility of employing propagule-free culture filtrate in screening of pepper varieties against *P. palmivora*. This is in conformity with the work of Lee (1973), who reported that the culture filtrate containing the toxic metabolite of the pathogen could be used as a tool for screening large number of *P. nigrum* types. The ability of *Phytophthora* spp to produce toxic metabolite *in vitro* was proved by few other workers also (Wolf, 1953; Ronnebeck, 1956; Savel'eve and Rubin, 1963; Sidel, 1961).

As the pathogen infects different parts of the plant, namely, leaves, stems and roots, the reaction of the pathogen on the different susceptible part has to be studied. Holliday and Mowat (1963) and Turner (1971 ; 1973) tested roots and detached leaves of different cultivars of *P. nigrum* and *Piper* spp. to ascertain their relative susceptibility and resistance against *P. palmivora*. Leather (1967) and Alconero *et al.* (1972) used root inoculation to screen the cultivars of *P. nigrum* and *Piper* sp. Ruppel and Almeyda (1965); Sarma and Nambiar (1979) and Sarma *et al.* (1980) conducted screening of *P. nigrum* varieties and *Piper* spp. through root inoculation only.

In the present investigation, different plant parts, namely, detached and undetached leaves, stem cuttings and roots of cuttings were inoculated adopting traditional methods with culture disc and zoospore suspension. Apart from these, all plant parts were bio-assayed with propagule-free dialysed culture filtrate.

Both detached as well as undetached leaves were inoculated with culture disc. The symptom development 48 hours after inoculation was observed. The findings on this are in agreement with previous observations (Holliday and Mowat, 1963; Turner, 1971; Alconero et al. 1972 and Mammootty et al. 1980).

The propagule-free dialysed culture filtrate when administered on the upper surface of the leaves after slight pricking, produced the same symptom as that of the inoculation with culture disc. This clearly indicates that the propagule-free dialysed culture filtrate containing toxic metabolite of the pathogen can also be used to test the reaction of the leaves of P. nigrum instead of using viable propagule of the pathogen for this purpose.

Lee (1973) has conducted a comparative efficacy of toxic metabolite of the culture filtrate of the

pathogen and the traditional inoculation with pathogen. But his studies were restricted only to stem cuttings. Administration of the propagule-free dialysed culture filtrate for assaying the toxic metabolite of the pathogen on the leaves is the first attempt in P. nigrum. Some workers have used the culture filtrate of different species of Phytophthora for conducting leaf assays and they succeeded in getting the typical symptoms (Ronnebeck, 1956; Savel'eva and Rubin, 1963; Seidel, 1961).

Administration of propagule-free dialysed culture filtrate resulted in the development of lesions within 24 hours, while inoculation with viable pathogen showed symptom expression only after 48 hours. This is attributable to the possible time lag required for the pathogen for infection, establishment and colonisation within the host tissue. Since the toxic metabolite is introduced directly into the host tissue along with the culture filtrate, rapid diffusion of metabolite into the host cells is ensured. However, the expression of symptoms was rapid for the first two days only and there was no further development of symptoms after

four days. But in the case of viable pathogen, the development of necrotic lesion was slow in the beginning. It was very rapid after three days and on the fifth day the entire leaf became discoloured and decayed (Tables 3 and 4).

It is possible that unrestricted growth of the pathogen in the host tissue under congenial environment (Temperature 20 to 22°C and saturated humidity favoured the rapid development of lesions followed by complete destruction of the host tissue. In the case of propagule-free dialysed culture filtrate, the quantity of diffusable toxic substances was relatively lesser the quantity of propagule-free dialysed culture filtrate administered being 0.05 ml only. Hence the tissue destruction was also proportionately limited. On the fifth day after the administration of culture filtrate, there was no further development of necrotic area as compared to the area on the fourth day. This clearly indicates that the entire toxic metabolites available in the culture filtrate had already diffused into the tissues of the leaves within four days.

The pattern of lesion development on undetached pepper leaves consequent on inoculation with viable pathogen and dialysed propagule-free culture filtrate was almost similar to that of detached leaves. In the former case, the inoculated leaves were shed after five days.

A comparison between the development of necrotic area on detached and undetached leaves show that the lesion development was rapid and intense on detached leaves as compared to inoculation with viable pathogen. But such a difference could not be observed between detached and undetached leaves to which culture filtrate was administered (Table 7).

The slow development of lesion and comparatively smaller necrotic area observed on the undetached leaves is explicable on the basis of the host reaction and the resistance offered by the living plant tissues against the attack of the pathogen. When the toxic metabolite is directly introduced into the host tissue, the diffusion is rapid and there is not much chance for the host reaction. In contrast, the detached leaves consisted of partly

dead tissues which may not possess natural power of resistance resulting in faster growth of a facultative pathogen like P. palmivora. This in turn, leads to rapid development of lesions without any restriction.

All the seventeen types of P. nigrum tested in this study were found highly susceptible to leaf infection of the pathogen. Eventhough slight variation in symptom expression was noticed during the initial stages of inoculation with pathogen, no significant variation in lesion development could be observed after five days both in detached as well as in undetached leaves. Thus, the results clearly show that all the tested types were highly susceptible to the disease. As compared to the detached leaves, the undetached leaves will give better indication on the resistance against the disease. In the former case, the natural resistance of the host is not manifested as the lesion development is very fast and unrestricted as compared to the latter. Therefore, it is reasonable to conclude that undetached leaf will be much better to study the resistance against the disease.

The development of symptom on stem cuttings when inoculated with zoospore suspension and placed in propagule-free dialysed culture filtrate was more or less same. This result supports the earlier finding of Lee (1973).

As in the case of leaves, the lesion development was rather slow during the first three days when the stem cuttings were inoculated with zoospore suspension. But it was faster till fourth day when the cuttings were placed in culture filtrate. Here again, the extent of lesion development was more in the case of propagule-free dialysed culture filtrate than the viable pathogen (Table 10). The faster and larger lesion development when the cuttings were placed in the culture filtrate can be explained by the fact that the availability of reasonably high quantity of the culture filtrate (100 ml) might have resulted in a continuous translocation and diffusion of the toxic metabolites from the culture filtrates into the host tissue.

All the seventeen *P. nigrum* types tested for stem reaction showed that there is no significant difference between the types in respect of symptom expression. All the types tested were highly

susceptible. Between the types tested, even slight difference in the tolerance was not revealed in the present study, perhaps due to the high load of zoospore suspension used for inoculation and the high quantity of propagule-free culture filtrate used for bio-assay. To get a tolerance limit among the susceptible types, it may require dilution and reduction of the quantity of the zoospore suspension as well as the culture filtrate.

In the case of root inoculation, symptom expression was unique with both viable pathogen as well as propagule-free dialysed culture filtrate. However, the symptom development was very fast when the roots were fed with propagule-free culture filtrate compared to inoculation with zoospore suspension (Tables 11 and 12). Here also, unrestricted lesion development were observed when the roots were fed with propagule-free dialysed culture filtrate and the entire roots were decayed on the fourth day. But in the case of inoculation with zoospore suspension, the time taken for complete destruction of roots was six days.

The relative difference in the lesion development on the roots in the case of pathogen and culture filtrate is attributable to the earlier explanation offered in the case of stem inoculations. Here also, the quantity of toxic metabolite of the pathogen fed to the roots was very high (250 ml) and the roots quickly absorbed the toxic metabolite and the entire root system was damaged within four days. Root being a much better absorbing organ it can be reasonably expected that more quantity of toxic metabolite present in the culture filtrate might have translocated and diffused into the root tissues than to the tissues of leaves and stem.

In both the cases, the entire roots were found to be decayed, within three days after administration of propagule-free dialysed culture filtrate or within five days when inoculated with zoospore suspension.

In all the pepper types tested by both methods, the entire roots were found decayed within three days after the administration of propagule-free dialysed culture filtrate and within five days when inoculated with zoospore suspension. This shows

that all the types tested were highly susceptible to root infection. After giving a vigorous test with high load of inoculum and high quantity of toxic metabolite, a comparative assessment of the susceptibility of different pepper types cannot be obtained within a short period.

In the light of the above results it can be concluded that the high concentration and quantity of zoospore suspension and the culture filtrate used for inoculation and assay may impose practical difficulties in screening the pepper types, since the precise degree of susceptibility cannot be detected due to the complete damage of entire roots, within five days and three days respectively.

The results discussed so far thus highlight that for obtaining a precise degree of host reaction to the quick wilt disease incited by P. palmivora more dilute zoospore suspension and culture filtrate would be required for screening large number of susceptible pepper types.

Summary

SUMMARY

1. *Phytophthora palmivora*, the causal agent of quick wilt disease of *Piper nigrum* is capable of producing toxin *in vitro*.

2. The leaves of the *Piper* plant assayed with propagule-free dialysed culture filtrate produced necrotic spots which are quite typical of the natural and artificial infections of *P. palmivora*.

3. Out of the five liquid media tried for the maximum production of toxic metabolite *in vitro*, Richards + yeast extract broth was found to be the best followed by potato dextrose broth and thiamine enriched synthetic liquid medium for *Phytophthora*.

4. Seventeen pepper types (open pollinated, hybrid and Panniyur-1) were screened against *P. palmivora* using viable inoculum and propagule-free dialysed culture filtrate.

5. Three different vulnerable parts of the pepper plant, namely, leaf, stem and root were subjected to screening.

6. On leaf, both viable pathogen and propagule-free dialy ed culture filtrate produced typical symptoms of

leaf infection consequent on inoculation or administration.

7. The initial symptom development was slow in the case of viable pathogen on leaf, while the same was rapid when the culture filtrate was administered.

8. All the tested pepper types showed high degree of susceptibility to leaf infection and there was no difference between the types on this respect.

9. Stem inoculation with zoospore suspension and placing the stem cuttings in propagule-free dialysed culture filtrate produced lesions typical of natural infection.

10. All the tested types were found to be highly susceptible to stem infection and there was no significant difference in the lesion development.

11. The one year old rooted cuttings when inoculated with zoospore suspension and fed with propagule-free dialysed culture filtrate, also showed typical lesions similar to natural infection.

12. Lesion development was fast in the case of propagule-free dialysed culture filtrate and the entire roots were decayed within three days.

13. Lesion development when inoculated with the zoospore suspension was rather slow as compared to the propagule-free dialysed culture filtrate but the entire roots were decayed within five days.

14. None of the seventeen pepper types tested in both the methods showed any degree of tolerance to the disease and there was no significant difference between the types tested for the development of lesion on the roots.

15. If used in the correct manner the propagule-free dialysed culture filtrate can be used for screening different pepper types to ascertain their tolerance to the disease.

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QUICK WILT DISEASE OF PEPPER-II

THE TECHNIQUES FOR SCREENING PEPPER VARIETIES AGAINST QUICK WILT DISEASE CAUSED BY PHYTOPHTHORA PALMIVORA (BUTLER) BUTLER

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study on the quick wilt disease of pepper (*Piper nigrum* Lin.) was conducted at the College of Horticulture, Vellanikkara.

The objective of this study is to find out a rapid and perfect technique to screen large number of *Piper* types (both open pollinated and hybrid seedlings) against *Phytophthora palmivora* (Butler) Butler, the quick wilt (foot rot) organism of black pepper.

P. palmivora is capable of producing phytotoxin *in vitro*.

The leaves of *P. nigrum* assayed with the propagule-free dialysed culture filtrate of *P. palmivora*, produced necrotic symptoms typical of the natural infection.

Five liquid media were tested for the production of toxic metabolite by the pathogen. Out of this, Richards + yeast extract broth was found to be the best medium followed by potato dextrose broth which is on par with thiamine enriched synthetic liquid medium for *Phytophthora*.

Seventeen *P. nigrum* types (open pollinated, hybrid and Panniyur-1) were screened against *P. palmivora*.

Three different plant parts namely, leaves (both detached and undetached), stem cuttings and roots were inoculated with viable pathogen and bio-assayed with

propagule-free dialysed culture filtrate.

The inoculation of the pathogen and administration of propagule-free dialysed culture filtrate on the leaves of potted pepper plants and detached leaves showed the same symptom expression as that of the natural infection of the pathogen.

All the pepper types tested were found highly susceptible to leaf infection.

Undetached leaves were found better to study the resistance of pepper plants to the disease, due to slow and steady development of symptom on inoculation with the pathogen.

Stem cuttings of all the P. nigrum types were inoculated with zoospore suspension and bio-assayed with propagule-free dialysed culture filtrate. The lesion development in both cases were typical of the natural infection of the pathogen.

All the pepper types tested for the stem reaction showed no significant difference between the types, with respect to the symptom expression.

The roots of cuttings were inoculated with zoospore suspension and assayed with propagule-free dialysed culture filtrate.

In all the pepper types tested by both methods, the entire root system were found decayed within three days after the administration of culture filtrate and within five days on inoculation with zoospore suspension.

All the seventeen P. nigrum types tested were found highly susceptible to root infection.