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Phytophthora spp. – A perpetual scourge in betelvine orchards

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Phytophthora causes enormous economic losses on crops worldwide, as well as environmental damage in natural ecosystems. Of which foot rot and leaf rot of betelvine (*Piper betle* L.) causes maximum damage in betelvine crop under West Bengal condition. The disease is produced by a number of species of *Phytophthora* includes *P.parasitica*, *P.palmivora*, *P.capsici.* In this paper scientific works of different researchers regarding nomenclature, symptoms of diseases, survival of *Phytophthora* spp. in soils of betelvine garden were presented. Epidemiological studies and management of diseases were also discussed. Different management studies as *in vitro* and *in vivo* effect of chemicals against *Phytophthora* sp., biological control and integrated management of *Phytophthora* rots of betelvine were reviewed.

Key words : Betelvine, *Phytophthora,* foot rot, leaf rot

INTRODUCTION

Phytophthora (from Greek word "phytón" means "plant" and "phthorá" means "destruction";i.e. "the plant-destroyer") is a genus of plant-damaging Oomycetes (water molds), whose member species are capable of causing enormous economic losses on crops worldwide, as well as environmental damage in natural ecosystems. The cell wall of *Phytophthora* is made up of cellulose. Approximately 100 species have been described, although 100–500 undiscovered *Phytophthora* species are estimated to exist. *Phytophthora* spp. is mostly pathogens of dicotyledons and are relatively host-specific parasites. Many species of *Phytophthora* are plant pathogens of considerable economic importance.

Phytophthora infestans was the infective agent of the potato blight that caused the Great Irish Famine (1845-1849), and still remains the most destructive pathogen of solanaceous crops, including tomato and potato. The soyabean root and stem rot agent, *Phytophthora sojae*, has also caused long standing problems for the agricultural industry. In general, plant diseases caused by this genus are difficult to control chemically, and thus the growth of resistant cultivars is the main management strategy. Other important *Phytophthora* diseases are: *Phytophthora agathidicide* Weir *et al*,—causes collar-rot on New Zealand kauri (*Agathis australis*), New Zealand's most voluminous tree, an otherwise successful survivor of the Jurassic age. *Phytophthora cactorum* (Lebert and Cohn) J. Schr'o't—causes rhododendron root rot affecting rhododendrons, azaleas etc. and causes bleeding canker in hardwood trees. *Phytophthora capsici* Leonian—infects Cucurbitaceous fruits, such as cucumbers and squash. *Phytophthora cinnamomi* var. cinnamomi Rands—causes cinna-

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mon root rot affecting woody ornamentals including arborvitae, azalea, Chamaecyparis, dogwood, forsythia, Fraser fir, hemlock, Japanese holly, juniper, Pieris, rhododendron, Taxus, white pine, American chestnut and Australian Jarrah. *Phytophthora fragariae* C.J. Hickman—causes red root rot affecting strawberries. *Phytophthora kernoviae* Brasier, Beales & S.A.Kirk—pathogen of beech and rhododendron, also occurring on other trees and shrubs including oak, and holm oak. First seen in Cornwall, UK, in 2003. *Phytophthora megakarya* Brasier and MJ Griffin—one of the cocoa black pod disease species, is invasive and probably responsible for the greatest Cocoa crop loss in Africa. *Phytophthora nicotianae* Breda de Haan—infects onions. *Phytophthora palmivora* (E.J. Butler) E.J. Butler —causes fruit rot in coconuts and betel nuts. *Phytophthora ramorum* Werres, De Cock and Man in't Veld—infects over 60 plant genera and over 100 host species; causes sudden oak death. *Phytophthora quercina* T Jung—causes oak death. *Phytophthora sojae*—causes soybean root rot.

Foot rot and Leaf rot of betelvine

Betelvine (*Piper betle* L.) is a perennial dioecious creeper, belonging to the family Piperaceae. It is an important commercial crop of India, Bangladesh, Sri Lanka and to some extend of Malaysia, Singapore, Thailand, Philippines and Papua New Guinea. In India it is commercially grown over an area of 54,000 ha providing livelihood to hundreds of thousands of families engaged in its cultivation and trade. Out of the total cultivable area under betelvine West Bengal constitute about 1/3 i.e. 18,203 ha. In West Bengal, betelvine is usually grown under artificially erected structure known as a Baroj, Bareja or Bheet, which is a kind of hut whose sides and roof are made of jute slaths or straw on a light frame work of bamboo which provides high moist and humid conditions favouring several diseases and are prerequisites for good harvest of crop. The major constraint to cultivation of betelvine is its diseases that damages foot, stem, root and foliage. The serious diseases reported include a foot rot syndrome produced by a number of pathogens including *Phytophthora parasitica* var. *piperina*, *Phytophthora nicotianae* var *parasitica,* species of *Rhizoctonia, Pythium* and *Sclerotium rolfsii* and foliage diseases like leaf rot by *Phytophthora parasitica*, *Phytophthora palmivora*, leaf spot and stem anthracnose caused by *Colletorichum capsici* and bacterial leaf spot and stem rot caused by *Xanthomonas campestries* pv. *betlicola*. Among the pathogens, *Phytophthora* sp. perhaps ranks first in its destructiveness under both field and storage conditions. The extent of losses may vary from 30 – 100% in case of foot rot and 20 – 40% in case of leaf rot, leading to almost total crop failure (Sengupta,2010; Datta, 2012; Dasgupta *et al*, 2015).

Phytophthora parasitica is the major fungal pathogen that causes qualitative and quantitative losses of betelvine (*Piper betle* L.). Among the soil borne pathogens attacking betelvine, *Phytophthora* sp. is wide spread in occurrence and probably most devastating. Incidence of two rots (foot rot and leaf rot) has been recorded in almost all betelvine plantations.

History and geographical distribution of **Phytophthora sp.** *in betelvine*

The disease in focus has been reported from almost all betelvine growing countries in the world including Indonesia, Mayanmar, Sri Lanka, Bangladesh etc. (Datta,2012).

The most destructive fungus is the foot rot caused by *Phytophthora* spp. The occurrence of foot rot caused by *Phytophthora parasitica* was noticed to be of destructive nature in Tamil Nadu, West Bengal, Uttar Pradesh and Assam. The disease was also noticed in Gupteshwar, Madhya Pradesh and in Mahoba, Uttar Pradesh.

In West Bengal, the foot rot and leaf rot of betelvine was recorded in highest intensity from Midnapore and Nadia districts (Dasgupta, 2015).

Symptomatology

In *Phytophthora* induced foot rot, wet rot associated with wilting of vines is common. In leaf rot circular, brown to black, water soaked spots developed on the leaves that enlarge fast extending the rot to the petiole and stems. Leaves subsequently are shed (Datta, 2012). Under intermittent wet and dry conditions clear zonations were seen on leaf giving a wavy appearance due to shrinkage or collapse of cells during intermittent dry phase. This disease was first identified from Maharashtra. The pathogen is a soil inhabitant but it perpetuates well in host tissues as dormant mycelium. Foot rot showed rotting at soil level. The foot rot is more prevalent during or just after rainy season while leaf rot occurs mainly during June to October because it is most common during or just after rains (Dasgupta, 2015).

Etiology and Nomenclature of Phytophthora sp.

There is a considerable confusion regarding the nomenclature of the species of *Phytophthora* causing diseases under consideration. The *Phytophthora* species reported to attack betelvine includes *P. palmivora, P. parasitica, P.capsici*. The isolates of *Phytophthora* collected from different locations of West Bengal were found to be the isolates of *Phytophthora parasitica* (Dasgupta,2015).

In spite of the *Phytophthora* sp. of betelvine has not been well investigated. The biggest problem facing the taxonomist is whether particular pathotype posses sufficiently stable new characters to a species novo or belongs to a subspecies classification. This dilemma will only be resolved when the extent of biological variation within each species is known. The problem of taxonomy is further compounded by the fact that *Phytophthora* species intercross readily, producing viable oospores.

Morphological characteristics of Phytophthora sp.

The available literature on species identity of the betelvine *Phytophthora* sp. showed a number of species appear to be involved e.g. *Phytophthora parasitica,* Dastur emend Ash, *Phytophthora nicotianae* Van Breda de Harn var. *parasitica* Watesh and *Phytophthora palmivora* (Bult). Both species *nicotianae* and *palmivora* produce papillate sporangia that were caduceus, heterothallic, amphigynous. Former one is close to *Phytophthora nicotianae* var. *parasitica* and the later is the part of *Phytophthora citrophthora*).

Datta (2012) collected several isolates from betelvine stems and leaves were grown in V_8 juice agar for initial mycelial growth and OMA for sporulation. The cultures of *Phytophthora* sp. showed that colony white or dirty white in colour moderately compact growth, dull white to milky white mycelial growth mostly with isolates but no aerial mycelium was found.

During morphometric study it was found that the average length and breadth of sporangia were 19.32-37.84 x 24.48-25.20mm. Highest length breadth ratio of sporangia was recorded 1.61:1 and lowest length breadth ratio was recorded 1.27: 1. The rest of the isolates with respect to L: B ratios were intermediary. Sporangial shapes were pear, lemon and spherical in shape. The largest chlamydospore was found 30.6m and smallest was 23.8m. Attachment of the chlamydospore was intercalary or / and terminal type. It was tentatively concluded that the species are closely resembled to *Phytophthora parasitica* (Datta, 2012).

Survival of Phytophthora sp.

Phytophthora species are not considered to be strong saprophytes and rarely survive in soil as mycelium if hosts or plant debris are removed from the field. Mycelium often survives by growing from year to year in living host tissues or in crop residues. However, *Phytophthora* has been found to persist in soil for at least 2 years after the death of trees in conifer forest. Other investigators have found that *Phytophthora* mycelium cannot survive over winter as a saprophyte.

There are conflicting reports on the value of chlamydospores as survival structures in the life cycle of this fungus. Mahanty *et al*, (2011a) worked on the effect of Irrigation Regime and IW: CPE Ratio on survival of *Phytophthora parasitica* (Dastur) causing foot rot and leaf rot of betelvine concluded that when interval of application of irrigation was increased, the survival of *P. parasitica* also increased and depth of irrigation had no significant effect on survival of the pathogen.

The survival of *Phytophthora* spp. was studied under controlled conditions with soil pH (5.4, 7.0 and 8.5), soil moisture (air dry, 25, 50 and 100% moisture level) and temperature (10, 20, 30 and 40°C) on survival ability of three isolates of *Phytophthora parasitica* (P21, P13, P8) causing foot rot and leaf rot of betelvine. The results showed that population of the pathogen increased up to first 2 weeks and then decreased gradually and disappeared within 13 weeks. This may be due to the germination of different spore forms and subsequent proliferation under natural conditions with or without food base. *Phytophthora* could survive more than 10 weeks at moderate soil moisture level (50%) and at a temperature range of 10-

30°C. High temperature of >40°C is unfavourable for the survival of *P. parasitica*. At 50% moisture, isolates 21 and 13 survived up to 13 weeks at 30°C, while at 10°C, all these isolates survived up to 14 weeks and at pH 5.4 all isolates had higher growth (Dasgupta *et al*,2012).

Seasonal variation in development of leaf rot disease of betelvine caused by Phytophthora **sp.**

Seasonal variation in development of leaf rot disease of betelvine caused by *Phytophthora* sp. revealed that the leaf rot disease existed during the monsoon months and it reached to the peak of severity during month of August to September depending upon the variety when the temperature and humidity remained within the boroj were maximum with heavy occurrence of rain. The disease severity became lower down with the advent of winter and less or no occurrence of rain i.e., from the month of end of October (Sardar, 2015).

Epidemiology of Phytophthora **sp.**

Epidemiological studies under field condition were carried out by stepwise multiple regression analysis of percent disease incidence of leaf and foot rot caused by *Phytophthora* sp on different varieties of betelvine (Bidhan Pan – I, Simurali Deshi and Simurali Gol Bhavna). The results of disease incidence for three years were compiled to find out the important meteorological parameters which were responsible for incidence of both the diseases. The results revealed that among the five considered meteorological factors, minimum temperature was more responsible for increasing the disease incidence or spread of the disease of foot rot and leaf rot disease in all the three varieties and in all the transformation models and in original data. In variety Simurali Deshi, in addition to minimum temperature and maximum RH was also responsible for increase in the disease incidence or spread of the disease of foot rot disease in all the transformation models. It was also revealed that among the two transformation models for disease prediction Logit transformation fit best for disease prediction and it confirmed high R^2 value. So, it was concluded that with increase in minimum temperature (› 26.5^0C) foot rot and leaf rot disease incidence increase significantly in case of Ghanagette and Simurali Gol Bhavna varieties whereas increase in minimum temperature and maximum relative humidity (96.5%) there was a significant increase in disease incidence of leaf rot of betelvine in Simurali Deshi variety (Datta *et al*,2009). The validation of the above results were made by further fitting the data against the disease incidence of foot rot and leaf rot of betelvine using the effective meteorological parameters of the same year for validation of these equations and models. The results showed poor estimated value during initial disease incidence. Among the two diseases (foot rot and leaf rot) the estimated value of leaf rot incidence was nearer to original value. It was observed that in all the prediction equations like Logit, Gompertz and Percent transformation, after 10th week of observations, the estimated value was similar to that of original value. It was more validated on leaf rot disease but not in foot rot disease. Among the three varieties tested, in Ghanagette and Simurali Deshi varieties, the prediction equations which were prepared also showed some validation in predicting the disease incidence of betelvine (Datta and Dasgupta, 2013). Datta (2012) compiled the results of 2006-07, 2007-08 and 2008-09 and validated with the year 2009-10 revealed that among the two diseases (foot rot and leaf rot) the estimated value of leaf rot incidence was nearer to original value. It was observed that in all prediction equations evaluated, after 8^{th} week of observations, the estimated value was similar to that of original value. It was more validated on leaf rot disease than foot rot disease. Further, results of validation during the year 2007-08, 2008-09 and 2009-10 with 2010-11 showed poor estimated value during initial disease incidence. Among the foot rot and leaf rot diseases the estimated value of foot rot incidence was nearer to original value. It was observed that in all prediction equations like Logit, Gompertz and Percent transformation, the estimated value was similar to that of original value in most of the observations (Datta, 2012; Dasgupta, 2013).

Management of Phytophthora Diseases of betelvine

Varietal resistance to Phytophthora **sp.**

Resistant cultivars in betelvine are very few because of cultivation of only male plants and difficulty in generating tissue cultured plants, due to the presence of various alkaloids there in. Under the AICRP on Betelvine, available varieties were screened at different AICRP centres under artificially created epiphytotic conditions for resistance to important diseases. Halisaharsanchi was found to possesses multiple resistance against *Phytophthora* rots. Pachaikodi and Karappu exhibited high resistance to *Phytophthora* rots (Dasgupta and Maiti,2008; Sardar,2015).

Cultural management

It is well established that a tiny amount of pesticide carried by the leaf would be hazardous to human health due to the residual toxicity of the pesticide as they are non-biodegradable and as the leaves are chewed directly immediately after harvest. Moreover microclimate inside the baroj favours plant diseases which depend upon the canopy structure of the baroj. An experiment was carried out for two consecutive years (2013 and 2014) to study the effect of different crop canopy by maintaining three different plant to plant spacing (viz. 11.1 cm. 9.53 cm. and 8.3 cm.) on leaf yield, disease incidence and keeping quality (days to 50% rotting) of betelvine. With the above plant to plant spacing and standard row to row spacing (60cm), the plant population was maintained as 1.50, 1.75 and 2.00 lakh ha^{-1}. The results revealed that when crop canopy was increased by reducing the plant to plant spacing from 11.1 cm to 8.3 cm there was significant increase in leaf rot disease. Significant increase in yield, decrease in fresh weight of 100 leaves and decrease in keeping quality of leaves were recorded when crop canopy was increased due to reduction in plant to plant spacing from 11.1 cm to 8.3 cm. From the results it can be concluded that microclimate developed in increase of canopy by reducing the plant spacing helped in spread and infection of disease incidence (Dasgupta and Sarkar,2016).

Chemical control

There is a considerable volume of literature on field trials starting from the early works of pioneers like Dastur, McRae, Hector, Chowdhury to present day. These have been recorded from time to time and the consensus that emerges is that no efficient method of controlling the *Phytophthora* diseases of betelvine is available till date, *i.e.,* effective as well as safe. The age old Bordeaux Mixture still has the highest promise.

Dasgupta and Maiti (2008) tested three promising fungicides (fosetyl-Al, chlorothalonil, Bordeaux Mixture) and phosphorus acid (Akomin) against 2 types of rots (root and foliage) caused by *Phytophthora* sp. The results showed that application of 1% Bordeaux Mixture at monthly intervals reduced disease intensity, application of 0.3% fosetyl-Al gave significantly higher leaf yield when applied at bimonthly intervals and 0.4% monthly application of phosphorus acid increased the fresh weight of leaves most significantly.

Mahanty and Dasgupta (2008) conducted a field trial for two consecutive years for the management of two different rots of betelvine caused by *Phytophthora parasitica* using four different fungicides (viz. Mancozeb, Fosetyl-Al, Chlorothalonil, Bordeaux Mixture) and phosphorus acid. The best treatment was Bordeaux Mixture. Applied at monthly interval it led to the lowest foot rot (8.19%) and leaf rot (10.74%) disease incidence. The 2^{nd} best treatment was application Fosetyl-al at monthly interval. The highest leaf yield and fresh weight of 100 leaves were obtained with Bordeaux Mixture application and this treatment also proved highly remunerative and was significantly better than all other treatments.

In field experiments, spray of mancozeb + carbendazim (0.2%) was found effective with 33.38% disease control (Patil *et al*, 2009).

Biological control

Of the two approaches of biological control viz. indirect through manipulation of associated micro biota through soil amendments and direct use of antagonists, the use of oil cakes in betel vine plantations is an age old practice. Similarly use of corn straws and til oil cake also reduced disease.

The effect of *Trichoderma viride* and plant extracts was tested against foot and leaf rot of *Piper betle* caused by *Phytophthora parasitica* var. *piperina* [*P. nicotianae* var. *parasitica*]. The spore suspension of *T. viride* (10^2 spores/ml), leaf extracts of *Azadiracta indica* (2%), *Pongamia pinnata* (3%) completely inhibited sporulation of pathogen whereas spore suspension of *T. viride* (10^4 spores/ml) and 4% concentration of leaf extracts of *A. indica, P. pinnata* completely stopped the mycelial growth of the fungus.

Sengupta *et al*, (2011) reported that four soil applications of *Trichoderma* sp. grown on in oil cakes at quarterly intervals, significantly effective in reducing the disease intensity (*viz.* leaf rot and foot rot of betelvine) and also increased the leaf yield. .Dasgupta *et al*, 2015 reported that bio control approach was not superior to chemical control, yet, it was at par in all aspects when biocontrol agent *Trichoderma* sp. were applied by mass multiplying in wheat grain, mustard oil cake and cowdung manure. Therefore, use of biological control agents is recommended to control foot and leaf rot of betelvine looking at the long term prospects and to avoid the possibility of health hazards due to consumption of toxic betel leaf.

Integrated Disease Management

Till the year 1999, very little information was published about the Integrated Disease Management (IDM) of betelvine. In this approach of management a promising combination of the fungicides/ pesticides along with the biocontrol agent was assessed. In few rows only chemical fungicides were applied and in other rows bioagents and fungicides were applied in combination.

Magdum *et al,* (2009) showed the minimum percent disease incidence (PDI) of foot rot and leaf rot was 6.84 and 1.85 respectively due to the treatment with sanitation + one soil application of 1.0% Bordeaux Mixture. The treatment also resulted in highest (58.93 lac/hac leaves) yield and C : B ratio (1 : 2.60).

Mahanty *et al*, (2011b) reported that integrated use of sanitation, Bordeaux mixture and *T. harzianum* gave the best control of foot rot and leaf rot of betelvine. The treatment yielded maximum leaf production ($3.448 x 10^6$ $ha^{-1} year^{-1}$) and the highest fresh weight of 100 leaves (351.85 g). The economic analysis showed that application of Bordeaux mixture along with *T. harzianum* and sanitation may be useful to obtain satisfactory control of both the diseases and economic benefit showing BCR of 1.48:1.

Sardar (2015) conducted the field trial by using biocontrol agents and Bordeaux mixture as drenching, spraying and drenching and spraying in combination revealed that the lowest disease incidence and disease index of both the diseases, highest yield and 100 leaf weight were recorded in treatment where Bordeaux mixture (1%) as soil drenching for 4 times and spray (0.5%) for 8 times were made. The highest C:B ratio was recorded where *Trichoderma* 0.5% as spray for 8 times were made.

When all the available control methods were integrated to control the diseases of betelvine it was found that the treatment consisting of application of Bordeaux Mixture at pre-monsoon + application of wheat grain containing bioagent one month after application of Bordeaux Mixture + application of Bordeaux mixture two months after application of Bordeaux mixture) gave the best result by inhibiting two types of rots, increasing the leaf weight and productivity of leaves. Economically it was also remunerative. That treatment may be of great practical implication due to its favourable effects under *in vivo* conditions. (Datta, 2012).

CONCLUSION

Although a lot of information regarding management of *Phytophthora* diseases of betel vine have been generated by the AICRP on Betelvine and different workers but till date all these are not enough to combat this dreaded disease of betelvine meaningfully, so it requires much more detailed study to make them fruitful to the farmers.

There are ample scope of research in the field of management of the *Phytophthora* diseases of betel vine. In IDM programme, the sources of initial inoculum need to be identified along with possibilities of their elimination. There are several reports that some co-lateral hosts harbour the pathogens. The irrigation water from the adjoining ponds may be the source of inoculum. The growers usually wash the infected material in nearby reservoirs and irrigate their crops with the same water, thus enabling the recurrence of the disease. So, the actual means of survival within and in the immediate vicinity of betelvine conservatory should be identified first and then factors affecting inoculum buildup and their dispersal need to be searched.

Investigations on the presence of different mating types of *Phytophthora sp.* in the infected betelvine gardens is very much essential because it may lead to the production of vigorous strains of *Phytophthora* sp. causing epidemic.

Trichoderma sp. was found to be an effective antagonist under *in-vitro* conditions. In the field of biocontrol agent, the use of genetically engineered strains (mutant) of *Trichoderma* sp. should be tried to over come the problems faced for the rhizosphere competence and less secretion of antifungal enzymes. The application of bio-protectants may be used on the foliage and also near the collar region which is very susceptible to infection by foot rot pathogens.

The management strategy thus obtained should be confirmed following *in situ* farmer's plot demonstrations.

Lastly it can be said that in the near future with the help of bio-technology disease free planting material and the varieties of betelvine, resistant to draught, flood and diseases should be possible for the benefit of growers.

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