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Survival of oospores and chlamydospores of *Phytophthora parasitica* var. *sesami* in plant debris

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Studies were carried out on viability of oospores and chlamydospores in plant debris for their role in carryover of the pathogen. Crushed plant parts (leaf and stem) of sesame infected with *Phytophthora* blight were placed in three different conditions viz. in ambient laboratory, in unsterilised soil in laboratory and in the field conditions. There was rapid biodegradation of the debris as well as loss in the viability of oospores and chlamydospores in comparison to the other two conditions during seven months of overwintering. Hence their role as source of primary inoculum of disease is not significant.

Key words: Plant debris, biodegradation, overwintering, *Phytophthora parasitica* var. *sesami*

INTRODUCTION

Plant pathogenic fungi may survive in various forms *viz*., collateral hosts, in soil or in seed to overwinter. The isolate of *Phytophthora* attacking sesamum (*P. parasitica* var. *sesami* Prasad) has been found to infect only sesame plant (*Sesamum indicum* L.) and does not form oospores either in culture or in the host tissues. *Phytophthora cinnamomi,* as per our understanding of this soil-borne pathogen interacts with plants and in the natural systems, and the ability to control the disease is limited. The pathogen has been found to be the cause of serious diseases in numerous species, a significant number of which are rare and threatened (Cahil *et al.* 2008).

Little is known about indigenous *Phytophthora* species in natural ecosystems. Increasing evidence, however, suggests that a diverse, trophically complex *Phytophthora* community is important in many forests (Hansen *et al.* 2012). Irwin and Mackie (2000) reported that *Phytophthora macrochlamydospora* produces large (up to 90 µm), thick-walled chlamydospores that distinguish it from all other Group VI species. In a series of growth cabinet, glasshouse and field experiments, tissue samples from living clonal *Eucalyptus marginata* (jarrah) were incubated immediately after sampling on agar (NARPH) selective for *Phytophthora*. *P. cinnamomi* was recovered 3–6 months after inoculation from 50% of samples with lesions and 30% of symptomless samples. However, up to 11% of samples with and without lesions and from which *P. cinnamomi* was not initially isolated contained viable pathogen (Huberli *et al.* 2000).

Soil and seeds have been considered as potential sources of primary inoculum in species of *Phytophthora* infecting many crops. McCarren (2005) appraised the role of chlamydospores as the main long-term survival propagules for *Phytophthora cinnamomi*. He presented the evidence for the formation of chlamydospores in nature, as well as differentiation between thin- and thick-walled chlamydospores, is examined. *P. cinnamomi* zoospores successfully colonised both root and leaf tissue of *Arabidopsis* and sporulation in the form of chlamydospores and sporangia occurred in leaves and roots of each ecotype but the number varied considerably between ecotypes (Robinson and Cahill 2003). Reports are available that the main source of infection of the black shank disease of tobacco caused by *P. parasitica* var. *nicotianae* is the tobacco plant debris left over from

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the preceding crop and the pathogen may live in the soil in the form of oospore as a saprophyte or in contaminated manure for five to six years. Species- specific protein was immunodetected only in *P. cinnamomi* samples, tested against total proteins from the same fungi grown on water with diagnostic bands of 55 kDa. The antiserum is therefore suitable for the specific identification of *P. cinnamomi* emerging in distilled water from infected tissues of chestnut, blueberry and azalea (Ferraris *et al*. 2004).

Phytophthora ramorum, Phytophthora alni, and *Phytophthora kernoviae* present significant threats to biosecurity. As zoosporic oomycetes, these plant pathogens may spread through natural waterways and irrigation systems (Kong *et al.* 2012). McCarren (2005) identified the gaps in our understanding of their behaviour in the natural environment, the length of time they survive dormant in soil and the factors that stimulate their germination. The ability of *Phytophthora cinnamomi* to survive long dry periods is the key to its persistence and are a significant long?term reservoir. In trees that died 12months after inoculation, *P. cinnamomi* was recovered from 60% of trunk and root core samples at 3months, declining to 33% at 10months, 5.5% at 12months and 0.1% at 34months after tree death (Collins *et al.* 2012). Perennation of oospores and chlamydospores in plant debris and their role as the primary source of inoculum of the blight disease has been reported in this article.

MATERIALS AND METHODS

Plant parts (Leaf and stem) of sesame infected with *Phytophthora* blight were collected separately during harvesting of the crop and cut into small pieces. The plant debris thus formed was collected separately in muslin clothes- as leaf pieces, stem pieces and leaf mixed with stem pieces. Five hundred of each category was tied in muslin cloth. One set of debris was stored in laboratory under ambient condition (control) and other sets were buried 7.5cm deep in earthen pots containing unsterilized field soil. These pots were kept under laboratory condition as well as in the field under natural condition (Lamari 2002).

Colour of debris, number of oospores, chlamydospores and their viabilitywere studied at monthly intervals upto seven months of their overwintering. The viability was tested by 2,3,5- triphenyl tetrazolium chloride (T.T.C.). Five milligrams of debris from each category was crushed and soaked in 5% aqueous solution of 2,3,5 - T.T.C. in a test tube for 2 h and kept in dark at room temperature (Lumsden 1980; Nelson and Olsen 1967). The test tube was shaken periodically. The suspension was observed under bright field microscope. Pink to red stained chlamydospores and oospores were considered as viable and unstained as nonviable.

RESULTS AND DISCUSSION

Initially, the fresh dried debris containing small pieces of leaf, stem appeared light green, off white to brown, but when the debris was buried in soil for seven months it underwent biodegradation forming very fine amorphous dark brown to black coloured powder in both laboratory and field conditions.

Debris kept in ambient laboratory condition

The viability of oospores and chlamydospores initially varied from 77-86% and 77-80% respectively in leaf,stem and leaf + stem mixed debris. Not much effect on their viability was seen after two months of storage, but later after seven months of storage (Fig.1A, 2A) it decreased to 53-58% (oospores) and 50-51% (chlamydospores).

Debris kept in unsterilised soil in laboratory

The different categories of debris buried in unsterilised soil showed 69-72% viable oospores and 64-60% viable chlamydospores. Their viability decreased gradually and became 49-52% and 42-46% respectively after seven months. The type of debris (leaf or stem) did not show significant difference in percent viability of oospores and chlamydospores (Fig. 1B, 2B).

Debris kept in field condition

Viability of both chlamydospores and oospores decreased sharply in the debris buried in soil under natural field condition. Initially, it ranged from 65-67% and 60-65% respectively for chlamydospores and oospores. After three months it decreased to 10-21% and 6-18%. Their viability decreased to 0-5% and 0-2% after five months. No viable oospores and chlamydospores were observed after seven months of overwintering.

In general, the percent viability was slightly high in oospores than the chlamydospores. Among the type of debris, mixed debris (leaf and stem) carried more

viable oospore and chlamydospores than the debris of leaf and stem used alone (Fig. 1C, 2C-D).

During the seven-month period of the study the debris underwent various changes. The biodegradation of debris was fast under field condition and

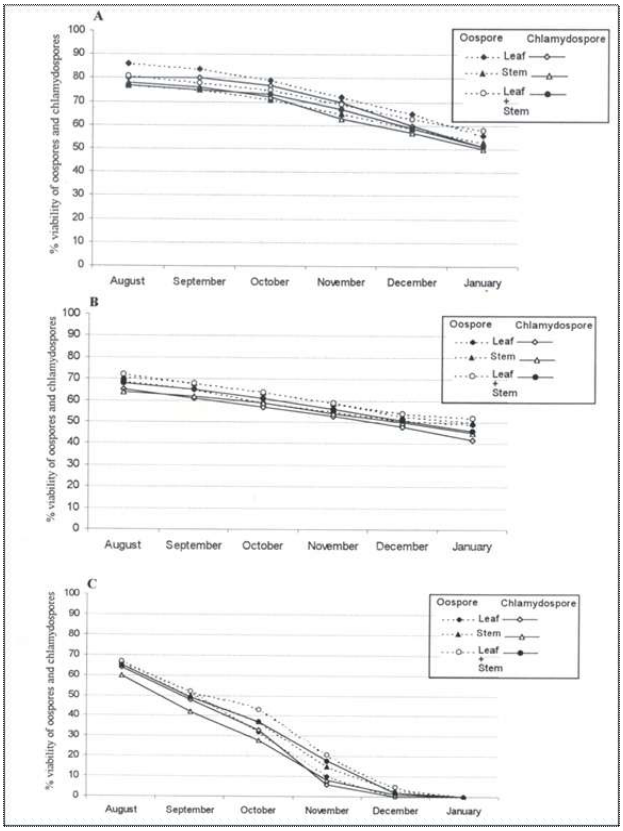

Fig. 1 : Percent viability of oospores and chlamydospores present in debris of leaf, stem and leaf+ stem mixed under ambient laboratory condition: (A) unsterilized soil inlaboratory, (B) and field condition, (C) of overwintering

within 2-3 months, it changed into amorphous dark brown powder. Not much degradation occurred in debris kept under laboratory conditions (both in ambient and in soil condition). Viability and germination capacity of chlamydosporesas well as oospores also declined rapidly under field condition than under laboratory condition. The viability of chlamydospores and oospores decline rapidly under field condition and almost all spores turned nonviable after 3-4 months of overwintering (Fig. 2C-D).

Since the debris was kept in soil in the month of July, it experienced high temperature and rainfall which increased the soil microflora causing degradation of debris and probably parasitized the chlamydospores and oospores of *P.parasitica* var. *sesami*. The inoculum however survived in uncontaminated conditions as provided in laboratory. Earlier reports of survival of oospores/ chlamydosporesupto five or six years are from the temperate regions of the world (Martin *et al.* 2012). But in Rajasthan, the survival of chlamydospores or oospores of *P. parasitica* var. *sesami* in debris in the drier sesame growing areas seems difficult. The thermal death point of *P. parasitica* var. *sesami i*s 48°C. It has also been reported that the survival of P*. parasitica* var *sesami* in soil is only up to one year and in a few districts of Rajasthan *viz*. Nagaur,

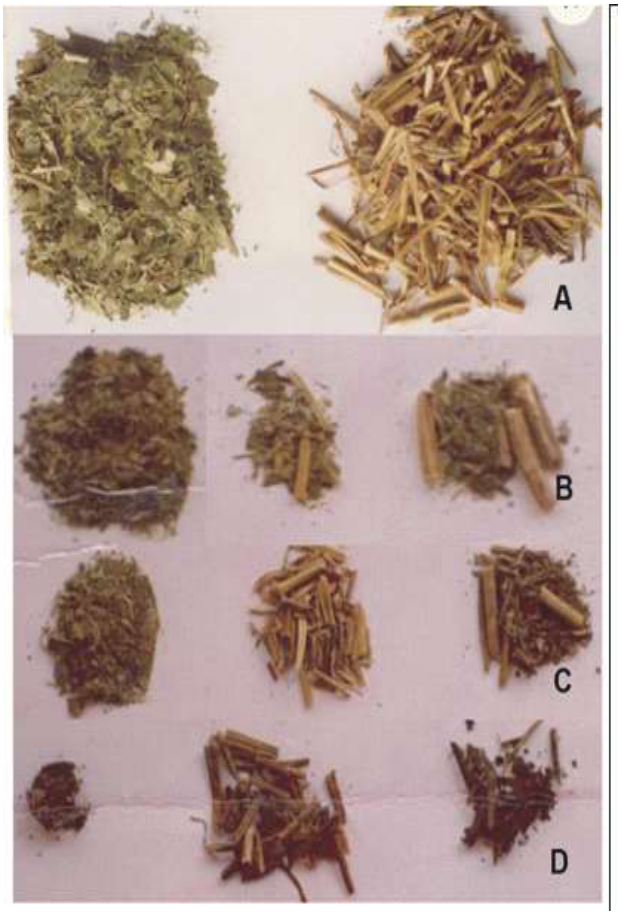

Fig. 2 : Debris of infected leaf and stem before and after overwintering to study the viability of chlamydospores and oospores under different conditions**:** (A) Fresh dried debris of leaf and stem. (B) Debris kept in ambient laboratory condition. (C,D) Debris showing biodegradation forming very fine amorphous dark brown to black coloured powder in both laboratory and natural field conditions.

Sikar, 'Pali, Jaipur, Tonk and Jalore. Except Nagaur all these districts have comparatively moderate conditions of temperature and rainfall as compared to drier districts of Rajasthan from where the seed samples were collected for the present study.

Saharan *et al. (*2017) reported that the conidial discharge decreases greatly from 12 noon to 8 p.m. Maximum conidial germination of *P. parasitica* from

B. juncea was at 20?C temperature. The germination of oospores of *P. parasitica* is dependent on temperature, light, pH of the medium, and age of oospores. Thus, in drier areas of Rajasthan, the only possible mode of carryover of pathogen and primary source of inoculum is the seeds, which are either infected or contaminated with mycelium, chlamydospores and oospores.

These tolerant germinants formed compact hyphae or secondary sporangia to allow longer survival of these pathogens. Long-term survival at a broad pH range suggests that these pathogens, especially *P. ramorum*, are adapted to an aquatic environment and pose a threat to new production areas through water dispersal (Kong *et al.* 2012).

In trees that died at 22 months, *P. cinnamomi* was recovered from 87% of trunk and root samples 2months after tree death, decreasing to 0.5% by 33months. This study suggests that the pathogen does not have a saprotrophic phase within dead *B. grandis* tissue, and is unlikely to be a long?term reservoir for *P. cinnamomic* (Collins *et al.* 2012).

Alternatively, the results were supported by Crone *et al.* (2013) as they suggested that asymptomatic, biotrophic growth of *P. cinnamomi* in some annual and herbaceous perennials and the production of a range of survival structures have implications for pathogen persistence over summer and its management. Similar observations demonstrated by Huberli *et al.* (2000) as by plating stem or bark tissue directly onto NARPH produced false-negative results for nine *P. cinnarnomi* isolates. The behaviour of the pathogen indicates that it could be present as dormant structures, such as chlamydospores, that need to be induced to germinate. These results have important implications for disease diagnosis and management, disease-free certification and quarantine clearance.

Dubey *et al.* (2011) reported the similar observation vivipary in our case might be due to fungal stimulation. In immature developing pods hyphae were observed in tissues of pericarp, placenta, locules and ovules. Such seeds when sown establish infection in the plants. Gaps are identified in our understanding of their behaviour in the natural environment, the length of time they survive dormant in soil and the factors that stimulate their germination. These are important issues that need to be addressed before we can adequately develop management strategies to control the spread and impact of *P. cinnamomi* (McCarren 2005).

Three ecological assemblages of forest *Phytophthora* species are hypothesized: aquatic opportunists, foliar pathogens, and soilborne fine-root and canker pathogens. Aggressive invasive species are associated with all three groups (Hansen *et al.* 2012). Meng *et al.* (2014) presented some recent progress of *P. parasitica* research by highlighting important features that make it emerge as a model species of oomycete pathogens. The emerged model pathogen will facilitate improved understanding of oomycete biology and pathology that are crucial to the development of novel disease-control strategies and improved disease-control measures.

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