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UNVEILING THE CLUBROOT PATHOGEN PLASMODIOPHORA BRASSICAE: INSIGHTS INTO ITS BIOLOGY, PATHOGENICITY, AND CONTROL STRATEGIES

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ABSTRACT
Clubroot is a disaster in the cultivation of crops of the Cruciferae family, caused by an obligate fungus, (*Plasmodiophora brassicae*). This pathogen survives in soil and crop debris for a long time in the form of a double-walled resting spore which is sub spherical to spherical in shape with 3 µm in diameter. Its severity is highest at a pH of 5.7, a cool temperature, and excess moisture. The biotic factors in its severity include the spore count in the soil and its virulence. Its dominant resting spore germinates to produce primary plasmodia. The primary plasmodia infect root cells, producing zoospores, which infect cortical cells and cause hypertrophy. This causes formation of typical club shaped galls in the roots. For its management, an integrated management system of agronomic, biological, and chemical approaches is required. Agronomic strategies include liming to raise pH, Boron application, crop rotation, cultivating resistant varieties, soil solarization, and sanitation. Similarly, biological strategies include use of microbial organisms like Trichoderma spp., *Gliocladium catenulatum, Streptomyces sp., Bacillus amyloliquefaciens,* and endophytes like *Acremonium alternatum* and *Heteroconium chaetospira.* Finally, chemical approach includes the use of fungicides like cyazofamid, Penta Chloro Nitro Benzene (PCNB), Nano Silver Hydrogen Peroxide, NF 48, thiophanate methyl, benomyl, and flusulfamide.

KEY WORDS
Crucifers, biopesticide, hypertrophy, structure, trichoderma, pathotyping, infection.

Plasmodiophora brassicae, a soil borne, obligate parasite, and a protist member of the supergroup Rhizaria, is the causative agent of clubroot in crucifers (Hwang, Strelkov, et al., 2012). It is neither a bacteria nor a fungus but shares the characteristics of both kingdoms (Ontario, n.d.). The kingdom Rhizaria has a common ancestry with Stramenophiles and Alveolata, which builds the SARP megagroup together with the kingdom Plantae (Burki et al., 2010; He et al., 2014; Schwelm et al., 2015). There are more than 300 species of plants as the hosts for *P. brassicae* (Karling, 1968); however the virulence of the pathogen isolates differs with different species (Honig, 1931a; Ren et al., 2016). The clubroot disease has been reported in more than 60 countries and has the potential to cause a 100% yield loss in crops (Dixon, 2009; Hwang, Cao, et al., 2012). Generally, in cauliflower, the loss in biomass ranges from 27-81% and the loss in curd yield ranges from 18-87%, but sometimes it can cause total failure of crop (R. D. Timila & Neupane, 2008). Besides the genus *Brassica,* this pathogen has been found to infect plants of the genera *Capsella* (Kim et al., 2011), *Raphanus, Arabidopsis, Eruca* (Paz Lima et al., 2004), and *Cardamine* (Tanaka et al., 1994). Moreover, it has also been found to infect plants of other families than the Brassicaceae. For example, it infects papaya (*Carica papaya*), clover (*Trifolium repens*), nasturtium (*Tropaeolum*), and corn poppy (*Papaver rhoeas*) (Ludwig-Müller et al., 1999; Ren et al., 2016). It has been noted to survive in the soil as a resting spore for 20 years in the soil without an economic host (Dasgupta et al., 1995).

The origin of the club root is still unknown, but the earlier literature suggests that the prevalence of club root disease was in the 13th century in Spain and later in the 17th century, when it was subsequently found in England, Scotland, and France (Rouxel & Regnault, 1985). According to (Dixon, 2009), the clubroot disease might be originated before 13the century at Roman times and ascribed to many causes. The Russian scientist Mikhail Woronin first identified this pathogen as the Plasmodiophorus organism and eventually

provided the name *Plasmodiophora brassicae* (Wikipedia, 2022; Woronin, 1878). In Nepal, the first incidence of the club root disease was observed in the Plant Pathology Division under the Nepal Agricultural Research Council (NARC) in 1993 from samples of broccoli, Knolkhol (PPD, 2000; R. Timila, 2006). Since 2004, widespread and severe epidemics of the clubroot pathogen have been seen in the production areas of Bhaktapur, Kathmandu, and Lalitpur districts (Timila et al., 2008).

For the development of clubroot disease on crops, various biotic and abiotic factors play a key role. According to (Agrios, 2005), a low pH of 5.7 provides a very conducive soil environment for the growth and development of *P. brassicae,* whereas its incidence is completely checked when the pH reaches 7.8. Other abiotic factors required for the development of this clubroot include mean air temperature between 18-23 °C, soil type, soil nutrient condition, and soil moisture at 70% with maximum water holding capacity and the biotic factors include its spore load and its virulence (Colhoun, 1953). The high spore concentration increases the percentage of clubbed plants rapidly at first, but the rate decreases as the diseased plant percentage increases (Macfarlane, 2008). The relationship between spore concentration, soil nutrient concentration, and rate of clubroot infestation is better explained by (Macfarlane, 2008).

The virulence of pathogens, within host multiplication, and within host transmission are the positively correlated traits in the pathogens (Frank, 1996), so higher levels and disease symptoms would conclude in the higher amount of within host reproduction rates (Botero-Ramírez et al., 2020; Sacristán & García-Arenal, 2008). On the other hand, (Murakami et al., 2004) found out that the number of resting spores produced by the pathogen is host-specific and hence, the severity of the disease symptoms are host-specific and cannot be attributed to the disease spore production alone. For instance, the resting spores per plant count in Chinese cabbage and broccoli plants at intermediate severity levels were 10^9 resting spores per plant, but cabbage plants with the same amount of disease severity produced only 10^8 resting spores per plant (Murakami et al., 2004). However, this disease decreases the aboveground herbivory of Bertha armyworm (*Mamestra configurata*) due to the induction of salicylic acid and its conjugates in affected plants (Weeraddana et al., 2020).

The roots of the infected plants become malformed due to excessive cell division and growth due to hypertrophy, which causes gall formation and ultimately blocks the pathway of water and nutrients to the aboveground parts of the plant (Encyclopaedia, n.d.). The symptoms appear in the underground parts of the plants. The clubroot symptoms are not the same in all the growth stages and vary according to the growth stage of the host plant, for example, the seedling stage may be characterized by aboveground wilting, stunting, and yellowing symptoms, whereas infection at later stages of crop life may not have any aboveground symptoms but it may cause them plants to senesce prematurely (Howard et al., 2010). Generally, the symptoms appear on the underground parts of the plants, but in severely infected plants, the symptoms can be seen at the aboveground parts, and hence, for assessing the clubroot symptoms, one should consider uprooting the plants and checking roots instead of shoots (Gahatraj et al., 2019). The infected plants have galls formed in their roots, which are club shaped. The galls or clubs formed on the root system may coalesce to form a gall complex in the root system, inhibiting the transport of water and minerals and resulting in symptoms such as stunting, wilting, and even premature death. These clubbings in the root system may be of five types: (i) the entire root system (main and lateral) gets clubbed, (ii) only the main root gets clubbed and lateral roots remain free, (iii) only lateral roots get clubbed while the main root remains free, (iv) clubs appearing as tumors as in radish, and (v) dark decomposed spots in the root system (Gahatraj et al., 2019). The symptoms may also differ with the host type. For instance, lighter plants like broccoli and cauliflower with root infections can have their hearts or spears deformed, while heavier plants like Brussels sprouts might get lodged (Howard et al., 2010). The symptoms that appear on the shoots might be confused with the symptoms of nutrient deficiency, other diseases, and lodging.

The genomic information about the pathogens from Rhizaria including *P. brassicae* is very restricted because of the fact that they colonize complex ecological niches and hence

the required amount of pure DNA is very difficult to retrieve (Stjelja et al., 2019). (Schwelm et al., 2015) published the genome of clubroot pathogen for the first time in history from the genomic DNA of resting spores of a single spore isolate e3, a European isolate (Javed et al., 2022). The genome of *P. brassicae* was found to be 24 Mb with the prediction of 9730 genes which had the support of seven transcriptome libraries and contained 4 introns per gene of length 60 base pairs (bp). The complete genome of e3 was published by (Stjelja et al., 2019) with the total size of 25.1 Mb which has helped a lot in the comparative study of genomics of Plasmodiophorids. Along with this isolate, five Canadian isolates (Rolfe et al., 2016) and a Chinese isolate (Bi et al., 2016) have been available for the study. The pathotype classification of these isolates is not easy due to their different differential hosts sets (Daval et al., 2019).

The genome of *P. brassicae* contains genes which could be potentially associated with the manipulation of host hormones such as isopentenyl-transferases, methyltransferase and cytokinin oxidase, and auxin-responsive Gretchen Hagen 3 (Schwelm et al., 2015). Like other eukaryotic biotrophic plant pathogens, *P. brassicae* also lacks several metabolic pathways (Daval et al., 2019). The missing genes in this pathogen encode proteins associated with arginine, thymine, lysine, and fatty acids biosynthesis pathways along with the proteins involved in sulfur and nitrogen uptake. Moreover, only few enzymes that are involved in the synthesis, transportation, and metabolism of carbohydrates are found in *P. brassicae* (Daval et al., 2019). Finally, the characterization of the *P. brassicae* is incomplete due to the limited number of genotypes and conditions from where above data are taken (Daval et al., 2019).

In 2009, the first clubroot-resistant (CR) canola cultivar became available to farmers, along with other cultivars from various seed companies. Genetic resistance has become the most important tool in Canada for managing clubroot, with 40 registered CR cultivars currently accessible. (Canola encyclopedia). Genetic resistance depends on single major genes that are efficacious against specific pathotypes or races of *P. brassicae*. (Rahman et al., 2014). A study also made by (Javed et al., 2022) focused on pathotyping, concluded that *P. brassicae* can be divided into pathotypes or races based on their prevailing virulence on divergent hosts. To understand the interrelation between *P. brassicae* and its hosts, five main pathotyping systems have been established. Currently, the Canadian clubroot differential, which involves a set of 13 hosts, is being used and has revealed 36 different pathotypes so far.

For proper clubroot identification, it is important to rapidly distinguish between different pathotypes, especially with the emergence of new pathotypes that can overcome resistance. Therefore, a different diagnostic approach is necessary for detecting pathotypes in clubroot. Currently, in bioassays with host differential sets, the responses of the hosts are observed based on the development of root galls, and *P. brassicae* pathotypes are phenotypically distinguished based on their virulence patterns. These tests are designed to measure the prevalence and degree of physiologic specialization in pathogen populations (Fredua-Agyeman et al., 2018). Apart from phenotypic approaches, microscopy also plays a role in pathotype identification. Various staining methods have been used under microscopy to observe the structure of *P. brassicae* and the host. Among these methods, the triple staining method is used to differentiate resting spores. (Buczacki & Moxham, 1979). Microscopy methods are ineffective for pathotyping because the cell morphology remains identical across different pathotypes. However, a PCR assay was developed for the detection of *P. brassicae* in soil. This assay is useful for identifying the presence of the pathogen and provides a more reliable and sensitive approach for pathotyping. (Ito et al., 1999). The primers used in the PCR assay were based on an isopentenyltransferase-like gene that is specific to *P. brassicae*. Additionally, another PCR assay was developed, which targets the internal transcribed spacer (ITS) region of ribosomal DNA, aiming to enhance sensitivity in pathogen detection (Faggian et al., 2007).

A different approach, known as metabarcoding, was studied for the detection of pathotypes present in P. brassicae. Metabarcoding utilizes Next Generation Sequencing (NGS), which provides high-quality single nucleotide resolution in a single reaction(Taberlet

et al., 2012). Metabarcoding works by extracting DNA from the sample, which is then subjected to an initial PCR to generate barcoded amplicons. These amplicons are subsequently prepared for next-generation sequencing. To identify the pathotypes present in the sample, the sequencing reads are aligned to the reference barcode database(Tso et al., 2021b). Hence, overall, molecular diagnostics approaches are considered fundamental for detecting pathotypes due to their high sensitivity, rapidity, and cost-effectiveness in terms of labor, space, and time. Additionally, these approaches do not pose biosecurity concerns and overcome the limitation of inter-rater reliability(Tso et al., 2021a).

Pathogenicity is the qualitative capacity of a parasite to infect and cause disease on a host, and virulence is the degree of damage caused to a host by parasitic infections, which is thought to be negatively correlated with host fitness (SACRISTÁN & GARCÍA-ARENAL, 2008). Because it maintains evolving new pathotypes and changes in the distribution and frequency of previously existing pathotypes within individual fields, the *P. brassicae* pathogen is remarkably variable in virulence. As a result, changes in the *P. brassicae* pathotype can overcome resistance in previously clubroot-resistant cultivars, resulting in unexpected disease outbreaks and additional yield losses (Zamani-Noor, 2017). *P. brassicae* isolates' virulence and physiologic specialization were first recognized in the 1930s (Honig, 1931b).

A research assessment of Clubroot disease severity collected from different Location found that disease incidence and disease severity percentage were significantly influenced by different pathotypes collected in a study of inoculated samples of cauliflower with different inoculum collected from different locations. At 65 DAI, seedling inoculated with inoculum collected from Kavre, Dhading, and Makwanpur districts had the highest disease incidence (100%), while seedling inoculated with Lalitpur had the lowest disease incidence (50%) (Ghimire et al., 2022).

Another experiment done on a series of greenhouse condition in order to assess the effect of *Plasmodiophora brassicae* virulence on clubroot development and resting spore propagation in 86 plant species from 19 botanical families concluded that P1 (+)-inoculated species had more severe symptoms (two to ten times more severe), larger galls (1.1 to 5.8 times heavier), and more resting spores than P1-inoculated plants. Hence, the emergence and spread of new virulence pathotypes of P. brassica capable of overcoming resistance highlights the significance of plant species selection in farming systems in clubroot-infected field (Zamani-Noor et al., 2022).

For the study of the life cycle of *P. brassicae,* several attempts were made to culture it on artificial media, but were not successful; however, the callus culture technique was used to study the life cycle of this pathogen where callus was generated from an infected root tissue of *Brassica spp.* (INGRAM, 1969; Tu et al., 2019). The callus culture done by (Tu et al., 2019) used Bulman dual culture system to prevent the confusion of other soil pathogen infection with that of *P. brassicae* infection and producing a contamination free culture of callus. The details of Bulman dual culture can be found in (Bulman et al., 2011).

The life cycle of *P. brassicae* is divided into three stages viz. resting spore stage, primary stage and secondary stage (Ingram & Tommerup, 1972). The resting spores are the primary inoculums, are sub-spherical to spherical in shape, and are about 3 µm in size (Kageyama & Asano, 2009). The dormant resting spores have refractile globules which might contain some materials for storage that is mobilized as the germination process of the spores starts (Macfarlane, 1970). The refractile globules dissolve and disappear after the onset of germination process where the dissolution starts from one side and spreads across the entire spore (Macfarlane, 1970). This paper finds out the presence of a small papilla which emerges from a pore in the spore wall of the resting spore; however, the relationship between the place where globules start to disappear and the formation of pore in the spore wall is undetermined. The spores do not germinate in in-vitro conditions below 20 ^{o}C but when seedlings are provided, they germinate in them, probably because seedlings provide necessary conditions for the spores to germinate and compensate for the low temperature (Chupp, 1917). The optimum temperature for the spores germination in in-vitro condition is 25 ^{o}C (Wellman, 1930).

After the resting spore germination, the primary zoospores, which constitute primary infection stage, are released which are 2.8–5.9 μm in diameter and spindle shaped with the presence of a pair of flagella (Ayers, 1944). They grow in size after their emergence from the resting spores, they fuse together, and become amoeboid when they reach the root cells of cruciferous plants: these amoeboid structures penetrate the cell walls of root cells and form young and separate thalli inside them of varying sizes where very small thalli form sporangia and very large thalli are transformed into zoosporangia arranged in compact irregular aggregations (Ayers, 1944). The infection of root hairs is favoured by high moisture (Ayers, 1944). The zoosporangia then form clusters in the root hairs and epidermal cells and then produce 4-16 secondary zoospores which penetrate into the cortical tissues initiating the secondary infection stage in cortical cells (Kageyama & Asano, 2009). They produce secondary plasmodia inside the cortical cells and induce hypertrophy causing gall formation in the roots (Kageyama & Asano, 2009). The plasmodia are finally developed into resting spores and released in the soil (Ikegami et al., 1982).

The spores of *Plasmodiophora brassicae* can be found over one meter deep in the soil and hence it is very difficult to eradicate the spores of this disease. Once the pathogen has been established, management strategies aimed at lowering disease incidence and severity and preventing crop losses are limited. At present, only small disease reductions have been obtained with chemical pesticides (Howard et al., 2010). Effective management of clubroot requires the implementation of an integrated disease management approach. No single control or disease management measure effectively prevents the infection process. Here are few of the management approaches that can be applied for clubroot disease:

1. Agronomic measures:

There are various agronomic practices that can be applied for the management of *Plasmodiophora brassicae*. This includes crop rotation, management of weeds and field sanitation, use of resistant varieties, seeding and planting time of the crops, soil health management, and use of trap crops. The clean and sanitized equipment and machinery, field sanitation, and removal of weeds from the fields can be a major step in slowing down the spread and development of the clubroot disease in the field (Javed et al., 2022). According to (Hwang et al., 2015), when susceptible canola crops were planted in rotation with non-host crops like Barley, Pea, and Oat, the clubroot severity and resting spore concentrations in the soil were decreased and yield increment was seen as compared with that of continuous cropping of either resistant or susceptible canola. For the best management of the clubroot pathogen, a standard recommendation of more than 2 years for crop rotation is provided (Hwang et al., 2019; Javed et al., 2022; Peng et al., 2015). Furthermore, the seeding and planting of cruciferous crops should be done at cold temperature since the high temperature is very conducive for the growth, development, and spread of clubroot pathogen (Feng et al., 2010; Gossen et al., 2012; Hwang, Cao, et al., 2012; Javed et al., 2022). The clubroot symptoms were seen less severe at temperatures greater than 26 °C and less than 17 °C (Gossen et al., 2012). The study done by (Hwang, Ahmed, et al., 2011) found out that more the seedling age during the transplantation is, the lesser is the clubroot severity. The most susceptible seedlings were of age 1 week, the seedlings with intermediate severity had age of 2 weeks, and the lowest severity of this disease was seen in the seedlings of age 3-4 weeks. Similarly, the soil sterilization should be done prior to the seeding and transplanting of crops to reduce the clubroot infestation. Thus, factors like soil sterilization, seedling age, soil and atmospheric temperature, and use of healthy seedlings of resistant varieties of crops should be considered while planting the crops to reduce the incidence of clubroot pathogen in the fields.

Similarly, soil pH is one of the most important factors for the clubroot disease development. Acidic conditions provide best environment for the development and spread of *P. brassicae,* so activities to increase the pH level of the soil like adding limestone, wood or ash, or calcium cyanamide, can be done for the prevention of this disease (Fox et al., 2022; Ghimire et al., 2022; Hennig et al., 2022; Hwang, Strelkov, et al., 2011; Javed et al., 2022; McGrann et al., 2016). The liming should be done at field hotspots and entrances in patches as liming the whole field is not considered an economical strategy (Donald & Porter, 2009;

Fox et al., 2022; Hwang, Strelkov, et al., 2011). Root hair infection and subsequent symptoms development were reduced upon increasing the pH above 7.2 with the application of cations (Ghimire et al., 2022; Myers & Campbell, 1985; Webster & Dixon, 1991). There are two types of lime amendments viz. slow acting and fact acting. Slow acting lime amendments like agricultural lime and dolomite lime should be applied at fall so as to provide enough time for them to break down and work whereas fast acting lime amendments like hydrated lime and quick lime can be applied at spring since they can work fast and increase soil pH at short time (Howard et al., 2010). However, the continuous application of lime in the field is not good, so soil testing is recommended prior to liming the field. Finally, the crops like *Phacelia,* black grass (*Alopecurus myosuroides*), field poppy (*Papaver rhoeas*), and field pea (*Pisum sativum*)can be used as bait or trap crops to ensure the germination of *P. brassicae* resting spores without affecting the main crop, eventually reducing the number of resting spores in the field (Ahmed et al., 2011; Javed et al., 2022; Zamani-Noor et al., 2022). The root exudates of the non-host bait crops stimulates the germination of resting spores of the pathogen (Ahmed et al., 2011; Friberg et al., 2005). The study conducted by (Friberg et al., 2006) found out that , leek (*Allium porrum*), winter rye (*Secale cereale*), and perennial ryegrass (*Lolium perenne*) significantly induced the resting spore germination of *P. brassicae*.

2. Biological Approach:

Unlike chemical method, biological method of clubroot management is environment friendly and helps not only to reduce the disease incidence, but also to maintain the fertility of the soil and a better soil environment to facilitate the growth and development of the other beneficial organisms. This method uses the locally available plant extracts, animal products, biofungicides, and micro-organisms for the disease management. Among various micro-organisms, most widely used micro-organisms in clubroot management in Asia, North America, and Latin America are *Trichoderma sp., Gliocladium catenulatum*, *Streptomyces sp.,* and *Bacillus sp.* (Botero-Ramírez et al., 2020; Diniz et al., 2017; Ghimire et al., 2022; Javed et al., 2022; Peng et al., 2014; Yu et al., 2015; Zhao et al., 2022; Zhu et al., 2020). A study conducted in Philippines with liming and Trichoderma application found that the clubroot incidence in cabbage reduced from 23% to 2% while the cabbage head weights increased from 0.75 kg to 1.45 kg (Cuevas & Bul-long, 2009). The commercial bio-fungicides like Serenade (Containing 1015.1 g/L *Bacillus amyloliquefaciens,* strain QST 713), Prestop which is now rebranded as Lalstop (*Gliocladium catenulatum* Strain J1446), Mycostop (containing mycelium and spores of *Streptomyces*, strain K61 actinobacterium, 5 x 108 cfu/g (cfu = colony forming unit)), Actinovate (*Streptomyces lydicus* WYEC 108), and Root shield (*Trichoderma harzianum* strain T-22), are available in the market that can be effectively used as biological approach for the management of clubroot of crucifers (Peng et al., 2014). These bio-fungicides have secondary metabolites which function against the clubroot pathogen. Moreover, micro-organisms found in these products colonize the rhizosphere and provide resistance to the plant against the pathogen through up-regulation of the genes which are particularly involved in the phenylpropanoid, jasmonic acid, and ethylene pathways (Lahlali et al., 2011, 2013; Lahlali & Peng, 2014; Peng et al., 2014). They do not reduce the germination or viability of the resting spores of the clubroot pathogen. Thus, they suppress clubroot infection by the primary and secondary zoospores in the root hair and cortical region simply by colonizing them and also reduce the colonization of cortex by plasmodia (Lahlali et al., 2013; Peng et al., 2014). An endophyte called *Acremonium alternatum* is also used for the clubroot management as it reduces the aboveground part of the plant healthier without considerable loss in the yield of Chinese cabbage, canola and arabidopsis; however, it does not reduce the symptoms in roots (Auer & Ludwig-Müller, 2015; Doan et al., 2010; Jäschke et al., 2010; Javed et al., 2022). According to (Jäschke et al., 2010), the endophyte *A. alternatgum* suppresses clubroot when inoculated before the clubroot pathogen or at the same time as with *P. brassicae;* the inoculation of the endophyte after the inoculation of clubroot pathogen is not effective. Similarly, *Heteroconium chaetospira* is also an endophyte which has shown to reduce the clubroot disease in the field plot of the *Brassica* plants (Jäschke et al., 2010; Narisawa et al., 1998). The bio-control agents work against the

pathogens through one or more of four ways, viz., competition, antibiotic production, parasitism/predation, and induced resistance/cross protection (Arie et al., 2001). Apart from these, cabbage manure has also shown promising results in the clubroot management (Ghimire et al., 2022).

3. Chemical Approach:

The seeds from the infected fields contain clubroot pathogen, which can be a mode for the disease transportation to a long distance; however, if the seeds are cleaned well and treated with chemical fungicides, they can contribute to the delay or prevention of clubroot infection (Hwang et al., 2014; Rennie et al., 2011; Rod, 1992). (Hwang, Strelkov, et al., 2011) studied the effects of various chemical fungicidal seed treatments like Prosper™ FX (clothianidin + carbathiin + trifloxystrobin + metalaxyl), Nebijin® (flusulfamide), Vitavax® RS (carbathiin + thiram), Dynasty® 100FS (azoxystrobin), and Helix Xtra® (thiamethoxam + difenconazole + metalaxyl + fludioxonil) in canola under field conditions and greenhouse conditions in western Canada. The study concluded that the seed treatments were very effective under greenhouse condition but had no significant impact on clubroot severity in heavily infested fields. The reason might be the insufficiency of these products to eliminate large number of resting spores in the surrounding environment, and the remaining spores would still be available for the infection (Hwang et al., 2014).

Not only the seed treatment, but the soil application of fungicides can also be done for the clubroot management (Hwang et al., 2014; Mitani et al., 2003). The cyazofamid, when applied to the field heavily infested with resting spores of *P. brassicae*, it was found that the root hair infection and club formation in Chinese cabbage were inhibited strongly (Mitani et al., 2003). The study suggested that the resting spore germination is directly inhibited by the fungicide cyazofamid, due to which the root hair infection and club formation are inhibited. It has also been speculated that cyazofamid inhibits the primary zoospore motility (Mitani et al., 2003). In addition, (Ghimire et al., 2022; Kowata-Dresch & May-De Mio, 2012) found Nebijin (flusulfamide) at the rate of 20 Lha^{-1} to be the best chemical control for clubroot of crucifers. Similarly, (Gahatraj et al., 2019) reported that the use of Terrachlor 75% WP i.e. PCNB (Penta Chloro Nitro Benzene) successfully decreased the mortality of seedlings and the severity of clubroot, and also increased the canopy cover and plant height in canola. (Buczacki & Cadd, 1976) found out that in glasshouse condition, the soil incorporation of NF 48, thiophanate methyl, and benomyl did well in the control of clubroot disease. The use of Nano Silver Hydrogen Peroxide induces Systemic Acquired Resistance (SAR) and limits the infection by the pathogen (Gahatraj et al., 2019).

CONCLUSION

Since the resting spores of *Plasmodiophora brassicae* persist long in soil and are mostly deep in the soil, the management of this disease is difficult and hence, it is considered one of the devastating diseases in cruciferous crops. Moreover, it can cause total crop failure. The pathogen has not been cultured in axenic media despite many efforts, and hence, infected callus culture has been used for the study of its life cycle. The resting spores germinate to produce primary zoospores which come in contact with the root hairs to cause primary infection. They reproduce in the root cells producing primary plasmodium which produces secondary zoospores and initiate the secondary infection stage in the cortical cells. This stage produces certain chemicals which induces hypertrophy and cause formation of galls in the roots. The aboveground symptoms of this disease include wilting, stunting, yellowing of leaves, and premature death of plants whereas underground symptoms include the formation of galls which block the pathway for water and nutrients from the roots to the upper parts of the plants. The aboveground symptoms appear at later stages of this disease and hence, for the identification of this disease at the earlier stages, one should consider uprooting the plants and see for the symptoms in the roots. For the management of this disease, no single method has been found highly effective. Thus, a combination of different strategies with agronomic, biological, and chemical strategies is required for its proper management.

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