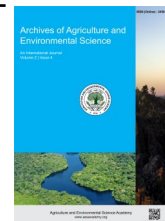




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REVIEW ARTICLE



## A review on clubroot of crucifers: symptoms, life-cycle of pathogen, factors affecting severity, and management strategies

Sachin Gahatraj<sup>1\*</sup> , Sundar Man Shrestha<sup>2</sup>, Tirtha Raj Devkota<sup>3</sup> and Harsha Hang Rai<sup>4</sup>

<sup>1</sup>Faculty of Agriculture, Agriculture and Forestry University (AFU), Rampur - 44209, Chitwan, NEPAL

<sup>2</sup>Department of Plant Pathology, Agriculture and Forestry University (AFU), Rampur - 44209, Chitwan, NEPAL

<sup>3</sup>Department of Horticulture, Agriculture and Forestry University (AFU), Rampur - 44209, Chitwan, NEPAL

<sup>4</sup>Ministry of Agriculture and Livestock Development (MoALD), Singh Durbar - 44600, Kathmandu, NEPAL

\*Corresponding author's E-mail: sachingtj19@gmail.com

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### ABSTRACT

Clubroot is a devastating disease of crucifers throughout the world. It is caused by a soil-borne obligate phytoparasite, *Plasmodiophora brassicae* Wor. Plant affected by this disease shows flagging of leaves, unthrifty growth, and even premature death. When uprooted, root shows characteristic symptom of hypertrophied club-shaped root system. Several biotic and abiotic factors affect the disease severity. Biotic factors include spore load in soil and virulence of pathogen, whereas abiotic factors generally include soil environmental factors such as soil temperature, soil pH, soil moisture, and soil type. Pathogen survives, for substantial period of time in absence of host, through its double-walled resting spores in soil or crop debris. Temperature affects spore germination, occurrence, and pathogen proliferation. Acidic soil reaction is crucial for pathogen to proliferate, metabolize, secret enzymes, and to complete life-cycle. All type of soil textures favor disease; however, severity differs with type of soil and soil organic matter content. Soil moisture provides platform to move bi-flagellated zoospores to infect root hairs of crops. Root hair infection is commensurate with inoculum density or spore load in soil. Immediate management strategies entail cultural practices, use of biocontrol agents, and application of chemical as last resort. *Trichoderma spp.*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Gliocladium catenulatum* are potential biocontrol agents. Flusalfamide, Fluzinam, and Cyazofamid are some common chemicals used to control clubroot. Soil carried by farm implements, human body, irrigation water, and flood can be potential source of pathogen. The risk of clubroot can be reduced by ensuring phyto-sanitary measures, destroying host crop debris, regular scouting, growing resistant cultivars, avoiding acidic soil reaction, eliminating weedy hosts, and reducing soil movement.

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### INTRODUCTION

Clubroot is potentially the most serious disease of crucifer crops, especially cabbage and closely related crops. It is caused by a soil-borne parasitic phytopathogen *Plasmodiophora brassicae* Woronin, which has already made economic impact in many regions of the world (Dixon, 2009). In 1878, Mikhail S. Woronin was first to recognize plasmodiophorous organism as causative

agent for clubroot disease, and gave name *Plasmodiophora brassicae* (Tommerup and Ingram, 1971). Later on, this disease has been reported in many countries of different continents such as India (Bhattacharya *et al.*, 2014), Australia (Donald *et al.*, 2006), America (Chittem *et al.*, 2014), Germany (Diederichsen *et al.*, 2014), and Canada (Rempel *et al.*, 2014). The plasmodiophorids have some common features such as zoospores with two anterior whiplash flagella of unequal length, long-lived

resting spores, biotrophic pathogens having multinucleated plasmodia (Braselton, 1995). The plasmodiophoraceae family consists of 10 genera and 35 species (Braselton, 1995). Heterogeneity for differential pathogenicity of *Plasmodiophora brassicae* Wor. has been found in clubs from same infested field or with in a club of same plant, showing that more than one pathotype may be present in a field (Jones et al., 1982). Initially, *Plasmodiophora brassicae* Wor. used to be considered to be an eukaryotic that belongs to kingdom Protozoa, phylum Cercozoa and sub-phylum Endomyxa (Cavalier-Smith, 1998). Later, the ribosomal RNA analysis of genus Plasmodiophora confirmed that it should be placed in the Kingdom Protist and phylum Plasmodiophoromycota (Castlebury and Domier, 2007).

It is almost impossible to grow brassica crops profitably in the disease appeared field. Infected plant is often totally losses. In general, *Plasmodiophora brassicae* Wor. is phytoparasite of crops and weeds of mustard family? There are all total 350 genera and 3700 species of herbaceous crops, weeds and ornamental plants (Dixon, 2009). Moreover, root hair infection also occurs in non-host plants such as lettuce (*Lactuca sativa*), ryegrass (*Lolium multiflorum*) and Spinach (*Spinacia oleracea*); however, clubs formation in root tissues does not occur (Murakami et al., 2002). In fact, root exudates of *Lolium perenne* stimulates germination of resting spores more than any other plants, including host brassica crops (Friberg et al., 2005) (Table 1). Short distance or local dissemination of spores is by drainage water, farm implements, wind-blown soil, animal movement, and most importantly seedling raised infested soil (Chai et al., 2014).

### Symptoms

The disease may progress to a considerable extent without showing any above ground visible symptoms. The earliest above ground symptoms are reduced development of plant, flagging of leaves, and wilting of entire plant in hot sunny days, as if the plant is suffering from water deficiency. It looks like wilting event when ample moisture is available in soil (Figure 1). However, the above ground symptoms are not sufficient to diagnose these plant as clubroot infected. We have to dig up the roots. When such plants are uprooted, the hypertrophied root system can be seen. The infected root form "club" shaped galls on main and lateral root system depending upon host species and nature of infection. Clubbing may be of several types: (a) clubbing of entire main and lateral root system as in cabbage, (b) clubs are present in only main root while lateral roots are free, (c) clubs are present in only in lateral roots while main root is free, (d) clubs appear as tumor as in radish, and (e) dark decomposed several spots in root system (Figure 2).

**Table 1.** Common host crops of *Plasmodiophora brassicae* Wor.

Common name	Botanical name	Family	Reference
Cabbage	<i>Brassica oleracea</i> L. var. capitata	Brassicaceae	(Cubeta et al., 2007; Lee et al., 2015)
Chinese cabbage	<i>Brassica rapa</i> L. chinensis	Brassicaceae	(Gao and Xu, 2014; Zhou et al., 2014)
Wild cabbage	<i>Brassica macrocarpa</i> Guss.	Brassicaceae	(Zhang et al., 2016)
Cauliflower	<i>Brassica oleracea</i> L. var. botrytis	Brassicaceae	(Kopecký et al., 2012; Kumar et al., 2019)
Radish	<i>Raphanus sativus</i> L. var. longipinnatus Bailey	Brassicaceae	(Kroll et al., 1984; Rowe, 2010)
Broccoli	<i>Brassica oleracea</i> L. italica	Brassicaceae	(Zhang et al., 2016)
Vegetable Mustard	<i>Brassica juncea</i> var. gemmifera	Brassicaceae	(Xue et al., 2017)
Canadian canola	<i>Brassica napus</i>	Brassicaceae	(Hwang et al., 2012)
Ryegrass	<i>Lolium perenne</i>	Poaceae	(Friberg et al., 2008)

Cytokinin from the plasmodium triggers the local initiation of cell division in the root cortex. As a result, a de-novo meristematic area is established that function as sink for host-derived indole-3-acetic acid (IAA), carbohydrates, nitrogen, and energy to maintain the pathogen and trigger the gall development (Devos et al., 2006). Cytokinin plays potential role in plasmodial development (Malinowski et al., 2016). Hypertrophy and hyperplasia both are responsible for gall formation. In cabbage, individual infections on root progress in both direction along with main axis and spindle shaped root is formed. The hypertrophy causes malfunctioning of xylem, which results flagging of leaves. The hypertrophy and hyperplasia of root tissue cause abnormal gall formation on roots, this impede water and nutrients translocation. Consequently, plant develops characteristics above-ground symptoms: wilting, stunting, yellowing and even premature death (Cao et al., 2009). Initially, plant wilts during mid-day, when sun-heat is intense, but appears healthy during morning. With accrument of severity, plant shows yellowing and stunting growth. In severe infection, precocious death of plant is undeniable.

### Life cycle of pathogen

*Plasmodiophora brassicae* Wor. has three stages in its life cycle: survival in soil as resting spore, root hair infection and cortical infection (Tommerup and Ingram, 1971). The life cycle of *P. brassicae* Wor. starts with resting spores. The pathogen persist in as resting spore in soil or crop debris. The resting spore has ability to survive for substantial period of time in soil, in absence of host crop. The resting spores, in soil, can germinate even after 17 years (Wallenhammar, 1996). The resting spores release primary zoospores which swim to the surface of root hairs where these penetrate through the cell wall. The primary zoospores are pyriform and biflagellate (Dring and Karling, 2007), with unequal biflagellum; one short with a blunt end and one longer with whiplash end. This stage is known as root hair infection stage (Kageyama and Asano, 2009). After this, pathogens form primary plasmodia inside root hair. Plasmodia undergo number of nuclear division synchronously; ultimately, plasmodia turns into zoosporangia. Now, each zoosporangium give 4–16 secondary zoospores which are released into the soil ready for the penetration of cortical tissue of the main root system, a process is called cortical infection (Kageyama and Asano, 2009). Later, pathogen develops into secondary plasmodia which are associated with cellular hypertrophy leading to gall formation of tissues. Finally, these plasmodia develop into new generation of resting spores that release into soil as overwintering structure (Kageyama and Asano, 2009) (Figure 3).



**Figure 1.** The above ground symptoms of clubroot observed in field; flagging of cabbage leaves in hot sunny day (Left) and unthrifty plant in severe form (Right) (Photo credit: Sachin Gahatraj, Agriculture and Forestry University, Nepal).



**Figure 2.** Clubroot of cabbage (Photo Credit: Sachin Gahatraj, Agriculture and Forestry University, Nepal).

### Factors affecting clubroot severity

The abundance and virulence pathogen, susceptibility of the host and suitability of environmental factors influence the severity of disease. When a plant is infected by a pathogen, environmental factors affect the severity of disease along with growth, development and yield of the plant.

#### Temperature

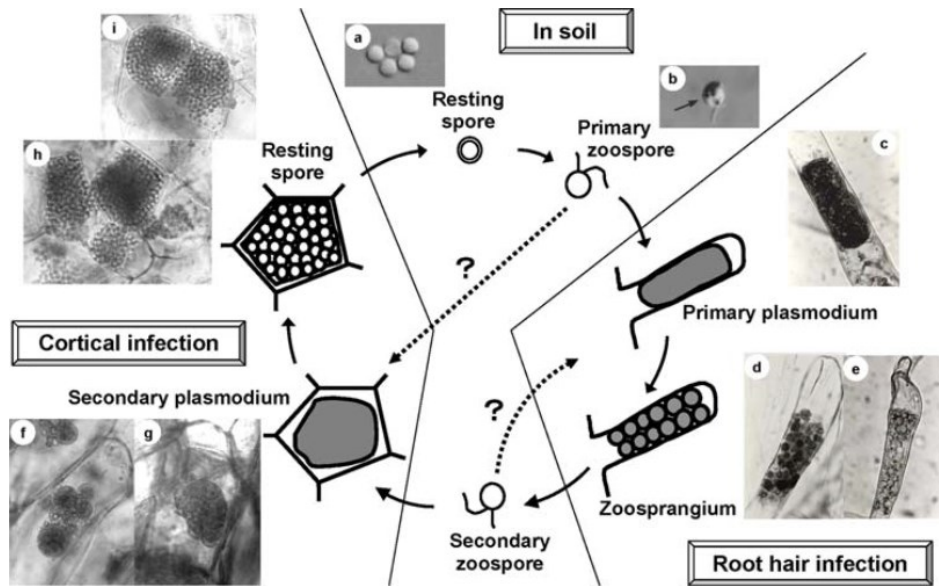
Temperature is a most influential abiotic factor; it affects spore germination, occurrence, survival, and development of pathogens, and ultimately, incidence and severity of disease they cause (Colhoun, 2003). Some diseases are most severe at low temperatures while others at high temperatures. In a research on Shanghai pak choi (*Brassica rapa* subsp. *chinensis*), temperature affected every stage of clubroot development; highest root hair infection and earliest visual symptoms initiation was at 25°C, intermediate at 30°C and lowest at 10°C. Root hair infection was observed at every temperature; however, characteristic symptoms developed only above 15°C (Sharma *et al.*, 2011). Above 15°C, the extent of cortical infection increased along with increased in temperature (Sharma *et al.*, 2011). In Asian countries, the damage caused by clubroot in Brassica vegetables can be minimized by seeding early spring and late summer in infested fields as clubroot incidence and severity were

highest for crop harvested in July-August and lowest for crops harvested in October because strongest positive correlation found between air temperature and disease severity over the last 10 days prior to harvest (McDonald and Westerveld, 2008).

#### Soil pH

The term pH (potential of hydrogen) is the measure of hydrogen ion [H<sup>+</sup> ion] concentration in a solution. It is expressed as the negative logarithm of hydrogen ion concentration and the scale ranges from 0-14. Higher H<sup>+</sup> ion concentration in solution leads to acidity (pH<7) and lower to alkalinity (pH>7) while pH = 7 denotes neutral (Buck *et al.*, 2002). The pH in their environment is crucial for microorganisms to proliferate, metabolize, secrete enzymes, and to complete lifecycle. At alkaline pH, reduction in clubroot severity has been observed as root hair infection by pathogen reduces at pH > 7 (Hamilton and Crete, 2010). In a field survey, in central Sweden, clubroot severity was high when the soil pH ranged 5.2 to 6.6 (Wallenhammar, 1996). Germination of resting spores and root hair infection are reduced by high soil pH is more than 7 (Dring and Karling, 2007) The spores of *Plasmodiophora brassicae* Woronin are either unable to germinate in alkaline soil, or have very low rate of survival and activity (Colhoun, 2003). The number of root hair infections decreased significantly when pH was increased from 6.2 to 7.1 (Niwa *et al.*, 2008).





**Figure 3.** Lifecycle of *Plasmodiophora brassicae* Woronin (Source: (Kageyama and Asano, 2009). **a** Resting spore. **b** Primary zoospores. **c** Primary plasmodia in root hair. **d** Zoosporangial cluster in root hair. **e** Empty zoosporangium. **f, g** secondary plasmodia in cortical cells. **h, i** Resting spores in cortical cells.

### Soil type

In addition to soil temperature and pH, soil texture has remarkable influence on clubroot occurrence and severity. All type of soils; light soil, sandy soil, clay soil, humus rich soil favor infection by *P. brassicae* Woronin, but level of clubroot is different even when different soils are inoculated with same spore load (Dring and Karling, 2007). The level of clubroot incidence and severity are reciprocal to amount of soil organic matter (Wallenhammar, 1996).

### Soil moisture

Soil moisture is an abiotic factor that affects the germination of spores, movement of zoospores as these are biflagellate (Braselton, 1995). Soil moisture serves as medium for biflagellated zoospores to move up to host root in order to infect it (Colhoun, 2003). Moreover, a slew of zoospores released when soil is moist or waterlogged. In contrary, sporangia do not release when soil is dry. Along with moderate soil temperature, adequate soil moisture is needed for development of severe clubroot and levels of clubroot severity is positively correlated with Orainfall (Gossen et al., 2012). A study showed that 60-70% soil moisture was the most favorable for clubroot development, but low level of infection can occur at low soil moisture and vice versa (Hamilton and Crete, 2010).

### Spore load

The root hair infection increases with increase in resting spores load of *P. brassicae* Woronin in the soil for Brassica crop such as cabbage and cauliflower. Incidence of root hair infection increased for two to three folds with increasing inoculum density as compared to increment in resistant cultivar (Hwang et al., 2011). The density of resting spore should be at least 1000 spores  $g^{-1}$  of dry soil for symptom development to occur and yield to be reduced in most of the Brassica crops (Donald and

Porter, 2009). After initial cultivation of Chinese cabbage, the resting spore densities were  $47.8 \times 10^5$  spores  $g^{-1}$  soil while  $1.6 \times 10^5$  spores  $g^{-1}$  soil after leaving fallow (Murakami et al., 2002).

### Current management options

Clubroot disease is responsible for tremendous yield loss of Brassica crops. On top of that, it is almost impossible to eradicate pathogen from field once get infested. Once field get infested, the management practices to reduce disease incidence and severity include raising pH through liming (Webster and Dixon, 1991), use of decoy plants to reduce pathogen spores load in soil (Murakami et al., 2002), inhibiting resting spores germination though neutral pH (Niwa et al., 2008), use of bio-control agents (Peng et al., 2011), Brassica vegetables harvesting date shifting to October when mean air temperature prior to harvest is appropriate (McDonald and Westerveld, 2008), reducing spore density in soil through crop rotation, fallowing, and nutrient amendment (Donald and Porter, 2009).

### Cultural strategy

Cultural practices like crop rotation and providing improved drainage conditions can protect crops from disease up to certain extent; however, when infested heavily, disease control generally is not satisfactory (Abbasi and Lazarovits, 2007). Some plants such as lettuce (*Lactuca sativa*), spinach (*Spinacea oleracea*) and Italian ryegrass (*Lolium multiflorum*) can be used to reduce root hair infection and clubroot disease severity (Murakami et al., 2002). The statement, prevention is far better than cure, best suits for this disease—once field get infested it is very difficult to make field pathogen free again. Prevention can be done by plying agricultural lime in fields with acidic soil reaction (Webster and Dixon, 1991). Moreover, neutral pH (pH = 7) inhibit germination of resting spores (Niwa et al., 2008).

Soil and crop management practices—such as crop rotation, fallowing, and nutrient amendment—can reduce spore density of pathogen in soil (Donald and Porter, 2009). For a single cycle, cropping of susceptible cultivar contributed  $2 \times 10^8$  resting spores  $g^{-1}$  soil while resistant cultivar contributed  $1.7 \times 10^7$  resting spores  $g^{-1}$  soil, compared to fallow soil (Hwang et al., 2011).

In addition, sanitation is an important aspect of clubroot prevention. Prevention of movement of resting spores to pathogen free field. As the spores of pathogen live in soil and movement occurs through soil mass, anything that can bring such soil into pathogen free soil as sources of the pathogen such as farm equipment's, tractor tyres, boots, tools, livestock, and containers. Soil can also move with flowing water. Soil from infested field can flow into water sources and other clean fields, which is often problematic during flooding. Soil moisture management, especially in root zone, plays pivotal role in clubroot management. High soil moisture means high chance of biflagellated zoospores to infect healthy plants. So, over-irrigation and water logging situation should be strictly avoided. Soil has capacity to hold, move, and infiltrate certain amount of water. It is important to improve soil physical properties to speed up infiltration of excess soil water in root zone. For this, soil need organic residue, reduced tillage, and alleviation of compaction. Host crop residue, especially root parts, acts as source of inoculum or pathogen reservoir. Hence, these should be removed from field and destroyed by burning after harvesting of economic plant parts. This practice gradually reduce spore load in soil. Boron when applied as borax (boric acid) is able to inhibit clubroot. Boron inhibits both primary and secondary stages of infection (Donald and Porter, 2009).

## Biological control

### *Trichoderma* spp.

Several species of *Trichoderma* have been reported as potential bio control agents against several soil-borne diseases caused by phytopathogenic fungi (Elad et al., 1987). *Trichoderma* (*Telomorph Hypocrea*) is a genus of fungi naturally found in many soil ecosystems among which some strains have ability to control severity of plant disease. It has ability to inhibit invasive plant pathogens through colonization in root intercellular spaces, antagonistic effects, mycoparasitic effects, competition, and inducing defense mechanism (Hermosa et al., 2012). *Trichoderma* reduced clubroot incidence by 45 % along with production of new roots allowing continuous development of crops and resulting higher crop yield (Cuevas et al., 2011). *Trichoderma* spp. suppress plant pathogenic fungi, and promote growth, increase plant height, leaf area along with increased yield of cabbage by 29% (Topolovec-Pintarić et al., 2013).

### *Pseudomonas fluorescens*

*Pseudomonas* spp. is ubiquitous bacteria in agricultural soils which are well suited as biocontrol agents for soil borne pathogens and growth promoting agents. The bacterization of seed

tubers with *Pseudomonas fluorescens* strains WCS374 lead to increased yield. Moreover, bacterization of wheat seeds with strain 2-79 increased yields of 17% in experimental plots and 11% in commercial plots (Weller, 2007). Several strains of *Pseudomonas fluorescens* such as; strains CHAO, Pf-5, Q2-87 and F113 have been able to synthesize antibiotics are effective in significant suppression of soil-borne plant pathogens through antibiotic synthesis such as; root rot of tobacco, *Pythium damping off* of cucumber (Weller, 2007). The fluorescent *Pseudomonads* belong to plant growth-promoting rhizobacteria (PGPR), which has remarkable capability of facilitating the plant growth of varied range of vegetables through nitrogen fixation, phosphate solubilization, mineral uptake and siderophore production. Moreover, it inhibits various phytopathogens through antibiosis and hydrolytic enzyme synthesis (Rai and Nabti, 2017). *Pseudomonas fluorescens* are reported as systemic resistance inducer against various soil-borne plant pathogens (Yan et al., 2007).

The application of *Pseudomonas fluorescens* and humic acid increased fresh biomass yield (46.8%), total biological yield (51.8%) and marketable yield (49.8%) over control (Maurya et al., 2017). The Plant growth traits in *Pseudomonas fluorescens*—denitrification and rhizosphere colonization—are responsible for enhancing growth and development of crops (Muriel et al., 2015). The anti-fungal metabolite, 2,4-diacetyl phloroglucinol, is present in *Pseudomonas fluorescens* which play pivotal role in the biocontrol activities (Delany et al., 2000). Treatment of ginger with *Pseudomonas fluorescens* strain EM85 along with solarization decreased *Ralstonia* wilt incidence to 7.42% and increased yield to 52.63% (Anith et al., 2000). *Pseudomonas fluorescens* was found effective in reducing the fruit rot disease of tomato as well.

### Others

Biocontrol agents, such as *Bacillus subtilis* and *Gliocladium catenulatum*, when formulated soil-drench is applied, reduced clubroot severity by more than 80% (Peng et al., 2011).

### Chemical control

The range of chemicals are fungicidal to fungal plant pathogens, but when used to manage *Plasmodiophora brassicae* Wor., a term "protozoicide" is more appropriate as this pathogen is not true fungi (Donald and Porter, 2009). Chemical pesticides should be used as a last resort when all other methods fail. Some chemical pesticides might be banned in one country, while not in others. Fluzinam and Cyazofamid are some effective in clubroot control; infect, these are more effective than biocontrol agents under high disease severity (Peng et al., 2011). Some of the commonly used chemicals against *Plasmodiophora brassicae* Wor. are as following:

### Nebijin (Flusulfamide)

Among dust and suspension formulation of flusulfamide (2',4-dichloro- $\alpha, \alpha, \alpha$ -trifluoro-4'-nitro-m-toluenesulfonamide), (Trade name: MTF651, Nebijin), suspension formulation was the most

effective; however, three rates (0.6, 0.9 and 1.2 Kg a.i. ha<sup>-1</sup>) were not significantly different. In contrast, 2.4 Kg a.i. ha<sup>-1</sup> of flusulfamide was found to cause a significant reduction in clubroot symptoms, incidence and severity (Cheah et al., 1998). The synthetic fungicides are attractive for clubroot control and mercury-based fungicides are most effective although these have environmental toxicity (Peng et al., 2014). The chemicals such as cyazofamid, fluazinam, flusulfamide, procymidone, prohexadione-calcium reduced clubroot severity through reduction in pathogen inoculum density and population composition, but results were not consistent (Peng et al., 2011).

### Penta Chloro Nitro Benzene (PCNB)

After the identification and evaluations of Penta Chloro Nitro Benzene (PCNB), it was reported that chlorinated nitrobenzene could result in substantial control under field that are not heavily infested with pathogen. In field trials conducted in Alberta, soil drench of PCNB (Terraclor 75% WP) reduced clubroot severity, seedling mortality and moreover, increased plant height and canopy cover of canola. In a glasshouse evaluation of chemicals, soil incorporation of benomyl, thiophanate methyl, and NF 48 found to be potential to control of clubroot (Buczacki and Cadd, 1976).

### Nano Silver Hydrogen Peroxide

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is byproduct of cellular metabolism, and it's largely produced in mitochondria, chloroplast, peroxisome, plasma membrane and cell wall (Quan et al., 2008). It induces PR 1 gene expression and systemic acquired resistance (SAR) in plant against pathogens (Neuenschwander et al., 1995). SAR is whole plant resistance response following earlier localized exposure to pathogen. In plant-pathogen interaction, hydrogen peroxide can limit the infection by pathogen by directly suppressing plant pathogen or inducing defense genes of plant cells. In addition, H<sub>2</sub>O<sub>2</sub> play pivotal role in regulating growth and development of plants (Quan et al., 2008). H<sub>2</sub>O<sub>2</sub> mediate stomatal closure induced by abscisic acid (ABA), which is an endogenous antitranspirant that reduce water loss from leaf surface avoiding wilting of plant (Tardieu and Davies, 2008).

### Alkylene bisdithiocarbamates

The numbers of alkylene bisdithiocarbamates related chemicals are active against clubroot. Among these, mancozeb (manganese ethylene bisdithiocarbamate) is one of the most effective (Buczacki and Cadd, 1976). Similarly, another alkylenebisdithiocarbamates related chemical, Zineb (zinc ethylene bisdithiocarbamate) was in use as alternative to benomyl transplant drenches, but this chemical showed phytotoxicity to cauliflower (Tate and Eales, 1982).

### Benzimidazole

Carbendazim is a broad-spectrum benzimidazole. It is a metabolite of benomyl, which is used as transplant drenches against *Plasmiodiophora brassicae* Woronin (Tate and Eales, 1982). Carbendazim (Methyl 1H-benzimidazol-2-ylcarbamate) was

found efficient for clubroot control only when used in a very high amount 80-100 Kg ha<sup>-1</sup>, but this makes treatment costly and soil polluted (Robak and Dobrzański, 2015).

### Conclusion

Tremendous yield loss in infection and persistency in soil for substantial period even in absence of suitable host make clubroot the major limiting abiotic factor of profitable brassica crops production worldwide. In absence of crop host and suitable environment, the pathogen (*Plasmiodiophora brassicae* Wor.) can survive in soil as resting spore for many years. Infected plant shows unhealthy growth, wilting during day, and stunted growth. Moreover, in case of severe infection, plant get yellowing and dies pre-maturely. Root system of infected plant is hypertrophied, typical club-shaped, and decayed on severe case—these are the major diagnostic symptoms of clubroot. The severity of clubroot disease depends upon soil environmental factors, such as soil temperature, soil pH, soil type, soil organic matter content, and spore density. It is better to forestall contamination of pathogen by plant quarantine, phytosanitation, sanitation of farm implements, liming of soil, soil nutrient amendment, regular scouting, etc. Even if field get contaminated with pathogen, disease severity can be gradually reduced by integrated management practices.

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### Conflict of interest

The authors declare that there are no conflict of interest regarding publication of this review paper.

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