PYTHIUM DISEASES, CONTROL AND MANAGEMENT STRATEGIES: A REVIEW

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ABSTRACT: Pythium species are morphologically polymorphic, physiologically unique and ecologically versatile, which make them significant both theoretically and practically. They are ubiquitous in soil and in water, distributed worldwide, and with very diverse host ranges. They include some of the most important and destructive plant pathogens, causing losses of seeds, pre-emergence and post-emergence damping-off, rots of seedlings, roots, basal stalks, decays of fruits and vegetables during cultivation, storage, transit or at the market, and cause serious damage of a wide variety of crops. The most aggressive species of Pythium that cause important plant diseases is P. aphanidermatum. It is a soil as well as seed born pathogen. The chemical fight and physical control of this fungal pathogen are very difficult to realize and in view of its broad host range, crop rotation also not completely control the pathogen. Moreover, use of chemicals to control the disease is also criticized throughout the world due to its detrimental effects on environment, as they are harmful for human and animal health as well as soil. Besides this, P. aphanidermatum became resistant to the common fungicides used against it. Eventually, it has forced scientist to find out the best alternatives to control this notorious pathogen. Hence here in this review paper the different control measures as well as use of plant extracts used against Pythium by researchers and many workers is summarized in brief.

Key Words: Pythium aphanidermatum, Plant Extracts, Antimicrobial activity, Pythium diseases

INTRODUCTION

The Pythium species are fungal-like organisms, commonly referred to as water molds, (kingdom Straminopila; phylum Oomycota; class Oomycetes; subclass Peronosporomycetidae; order Pythiales and family Pythiaceae) are worldwide in distribution and associated with a wide variety of habitats ranging from terrestrial or aquatic environments, in cultivated or fallow soils, in plants or animals, in saline or fresh water. The genus Pythium is one of the largest oomycete genus and consists of more than 130 recognized species which are isolated from different regions of the world [123, 46, 41, 120, 19, 139]. Although it generally is recognized that species of Pythium are not host specific. Most of the species are known to parasitize and cause infections in the crop plants and ultimately damage them [123, 85, 66, 83]. Pre- and post-emergence damping-off disease caused by Pythium spp. in vegetable crops is economically very important worldwide [182]. Rapid germination of sporangia of Pythium after exposure to exudates or volatiles from seeds or roots [108, 114] followed by immediate infection make management of Pythium very difficult [182]. Pythium diseases of vegetables and field crops are considered as important limiting factor in successful cultivation of crop plants throughout the world. It is estimated that diseases caused by Pythium species in different crops are responsible for losses of multibillion dollar worldwide [174].

Pythium aphanidermatum (Edson) Fitzp. is one of the most aggressive species in the genus and has a wide host range causes many economically important diseases and is one of the most pathogenic species in the genus. Its colonies on cornmeal agar (CMA) have a cottony aerial mycelium, on potato–carrot agar (PCA) it has some loose aerial mycelium without a special pattern and with heavy aerial mycelia on potato dextrose agar (PDA). Main hyphae are up to 10 µm wide. Zoosporangia consist of terminal complexes of swollen hyphal branches of varying length and up to 22 µm wide. Oogonia are terminal, globose, smooth and of 20–26 µm diameter. Antheridia are mostly intercalary, sometimes terminal, broadly sac-shaped, 11–15 µm long and 9–15 µm wide. Oospores are aplerotic [123, 181, 46].
Inoculum of *P. aphanidermatum* may enter in the crops through a number of sources including contaminated potting media [104]. Contaminated irrigation water can serve as an additional source of inoculum, particularly in flood irrigation in which drainage times are long [69, 146] *P. aphanidermatum* can survive as oospores, sporangia, and/or mycelium in organic debris on floors, soil and tools. [161, 104] *P. aphanidermatum* can also be spread by shore flies and fungus gnats. [55, 71]. *P. aphanidermatum* reduces vigor, quality and yield of crops, often killing a large percentage of plants affected. It is cosmopolitan in distribution and one of the most common plant parasitic pathogen of a number of different crop plants. *P. aphanidermatum* is an aggressive pathogen at high temperature (53). It is known to cause infection on a wide range of plant species, belonging to different families viz., *Amaranthaceae, Amaryllidaceae, Araceae, Basellaceae, Bromeliaceae, Cactaceae, Chenopodiaceae, Compositae, Coniferae, Convolvulaceae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Gramineae, Leguminosae, Linaceae, Malvaceae, Moraceae, Passifloraceae, Rosaceae, Solanaceae, Umbelliferae, Violaceae, Vitaceae, Zingiberaceae* [181].

The diseases caused by *P. aphanidermatum* varies with the host plant, it is the causal agent of pre and post emergence damping-off of various seedlings. It also causes seedling rots, root rot, cottony-leak, cottony blight, stalk rot etc. Moreover, new host and diseases caused by this destructive pathogen are continuously reported. Damping-off and root rot caused by the *P. aphanidermatum* are considered among the most devastating diseases of greenhouse-grown crops. A significant number of studies on *Pythium aphanidermatum* have been carried out in different parts of the world [123]. It affects nearly every crop grown in every part of the world [24].

It has also been isolated from different areas of the world such as root rot of vegetable pea in Australia (89), foot rot of ulluco in Japan [170], leaf rot of *A. fasciata* in China [140], rot on *Brassica campestris* chinensis group in Japan [166], damping-off, vascular wilt and root rot of groundnuts in Australia (23), damping-off of cauliflower in India [154], damping-off in fenugreek in India [101], rot of leaves, stems and roots of B. rubra in Japan [171], root and stem rot of poinsettia in Argentina [116], stem canker of *Amaranthus caudatus* in Argentina [109], cottony leak of scarlet runner bean in Japan [13], soft rot ginger in India [80], damping-off of cucumber in Oman (45), root and crown rot of melon plants in Honduras [33], root and crown necrosis of adult bean plants in Spain (149), *Pythium* rot of figmargold in Japan (81), rhizome rot of ginger in India [143], damping-off and root rot of soybean in USA [141], damping-off of chilli in India [145], damping-off of *Cucumis meloin* China [75], damping-off disease in tobacco seedbeds in India [162], watermelon "sudden death" of greenhouses in Spain [58], post-emergence damping-off in chili in India [106], rhizome rot disease to turmeric crops in India (128), damping-off disease of *Aquilaria agallocha* seedlings in India [164], root rot of turf grass in China [75] and damping-off of cabbage seedlings in Japan [84] etc.

In spite of its devastating effect, chemical fight [50] and physical control [25] of this fungal pathogen are very difficult to realize.

There are several agricultural practices followed by farmers to control diseases caused by this notorious pathogen such as crop rotation, use of resistant cultivars and planting disease free seeds but as the pathogen is not specific in host range and can survive well in every condition in the form of oospores and sporangia for long time period so these practices are found to be less significant, besides these practices physical methods i.e. soil solarization has been used for managing diseases caused by *Pythium* species. Soil solarization is a hydrothermal process that occurs in moist soil when the soil is covered by plastic film and heated by exposure to sunlight during the warm months. The process changes soil physical, chemical, and biological properties and thereby helps to improve soil health. It is an alternative to soil fumigant agricultural chemicals that have significant environmental risk, a negative impact on beneficial soil microorganisms, and that are not user friendly [136]. Efficacy of soil solarization against *Pythium* species has been reported by many workers, Katan [79] reported the efficacy of soil solarization in reducing the disease caused by *Pythium*. Usman [172] reported the significance of soil solarization against *Pythium* spp. Kusum Mathur [87] conducted soil solarization experiment in Rajasthan college of Agriculture, Udaipur, using thin transparent polystyrene film, and found significant reduction of *Pythium* spp. Soil solarization helps the farming community to manage the disease effectively and also enhance the yield level [57,73]. Deadman [44] reported that soil solarization is not only significant against *Pythium aphanidermatum* but enhance the vegetative growth of the crop also. No doubt that solarization appears to be an effective practice able to control soil borne pathogens, even though it may cause temporary stress on some beneficial soil microbial biomass [136].
Besides the physical method of soil solarization, organic materials were also used against disease caused by *Pythium* by many workers, Kao and Ko [78] Paulitz and Baker [121] Shuler [156] and Matsuzaki [98]. Boehm and Hoitink [29] and Theodore and Toribio, [168], Craft and Nelson [38], Boehm [29], Ringer [138] Erhart and Burian, [49] reported that there are many factors present in organic material which affects the suppression of disease, these factors include the quality and quantity and associated levels of microbial activity. Lumsden (90), Hadar and Mandelbaum [61], Mandelbaum [92], Mandelbaum and Hadar [91] and Teodore and Toribio [168] reported that sugarcane residues, poultry slurry and municipal bio-solids are also showed good disease suppressing activity against *Pythium aphanidermatum* and other *Pythium* species. However, solarizing soils along with suitable organic materials gives good result as compared to individual treatment of either soil solarization or use of organic materials. It has been reported that organic materials when added in the soil then it actuate a chain reaction of chemical and microbial degradation, which enhance toxicity against soil borne plant pathogens. The addition of organic manure amendment probably contributed to the higher nutrient contents and increases the yield [7, 51]. Satya [147] reported that composted chicken manure alone at 5381 kg/ha reduced populations of *Pythium* sp. significantly and when combined with heat (42°C), eradicated the *Pythium* population from the soil. Organic soil amendments can protect soil microbial biomass and enzymatic activities from the detrimental effect of heating which is caused by soil solarization [136]. The one more important method which is used against the *Pythium* besides soil solarization and organic amendment; is the use of biocontrol agents. Among the mechanisms that biocontrol agents use to control *Pythium* diseases are production of antagonistic metabolites, competition for space and nutrients, mycoparasitism, hyphal interactions, enzymes secretion and actually feeding on *Pythium* propagules. Elad [48] reported that *Trichoderma harzianum* was significant antagonist against *Pythium* species. Lumsden and Locke [90] reported that *Gliocladium virens* can significantly control the damping-off of zinnia, cotton, and cabbage caused by *Pythium ultimum*. The studies revealed that the *Trichoderma harzianum* was effective in reducing disease incidence and increasing crop germination [151]. Manasmoondhas and Sivaprakasam [94] and Manorajitham and Prakasam [95] reported the antagonistic activity of *T. hamatum*, *T. harzianum*, *T. reesai*, *T. viridae* and *Pseudomonas fluorescens* against *P. aphanidermatum*. Ram [133] reported that biocontrol agents *T. harzianum*, *T. aureoviride* and *T. virens* against *Pythium* gave significant reduction in the disease and also increased the yield. Bardin [21] reported that *Pseudomonas fluorescens* was significant in controlling *Pythium* damping-off of sugar beet. Talc based formulation of antagonist in reducing pre-and post emergence damping off of chilli caused by *P. aphanidermatum* was reported by Haritha (64). Kamala and Indira [77] were also reported that 32% out of 110 *Trichoderma* isolates were found to be strong antagonistic against *Pythium* species, *T. hamatum* and *T. harzianum* were reported to be significant against *Pythium aphanidermatum* and *Pythium ultimum* in chilli. Shweta [157] also reported that *T. harzianum*, *T. virens* and *Pseudomonas fluorescences* were found to be antagonistic against *Pythium*. Biocontrol agents work best when pathogen pressure is low to moderate, because their activities against pathogens are biological by nature, it is possible that they will not be effective when overwhelmed by high pathogen levels. In addition, biocontrol agents are generally not effective if once the plants have been infected by *Pythium* and thus should not be considered curative control treatments [21]. Besides all the agricultural practices and physical and biological methods for the control and management of diseases caused by *Pythium*, chemical fungicide is most commonly used by the growers. Mancozeb, Carbendazim, Ridomil and Topsynt etc has been recommended against *Pythium* [11, 12, 125, 154]. Such synthetic fungicides bring about the inhibition of pathogens either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective [115]. But in case of *Pythium* disease, if once the disease enters into the field, it is almost impossible to control properly by using only chemical fungicides. Moreover, use of chemicals to control the disease is also criticized throughout the world due to its detrimental effects on environment, as they are harmful for human and animal health as well as soil. They enter the food chain and cause several deleterious effects on biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification and compaction, thatch accumulation, and diminished resistance to diseases [155]. Moreover Inappropriate, overzealous and indiscriminate use of most of the synthetic fungicides not only imposes adverse effects on ecosystems, besides this, it also created a possible carcinogenic risk and toxicological problems higher than that of insecticides and herbicides put together [31, 115, 97, 59]. The range of effective registered chemical fungicides currently available to growers for managing *Pythium* diseases is narrow. Several of these fungicides are quite effective against *Pythium* diseases, though most involve single modes of action against the pathogen, however, the single mode of action can be defeated quickly by the development of resistance to them in *Pythium* populations promoted by widespread and intense use of them. The narrow list of available fungicides combined with the potential for rapid development of fungicide resistance and public concern for human and environmental health has made the disease management very important for growers to practise.
Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective [189]. Development of fungicide resistance by Pythium species further discourages its use for disease control [182]. Some populations of Pythium are reported to have resistance to metalaxyl, mefenoxam and/or propamocarb [103]. Certain fungicides, usually systemic fungicides, are said to be ‘at risk’ to the development of resistance if they are used repeatedly [21]. It is recommended that chemicals at high risk be used sparingly and in rotation or mixed with chemicals with different modes of actions. Besides all these Folman and his coworkers [50] reported that chemical fight against this aggressive pathogen is not significant. Hence there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals. Several researches have been conducted to control soft rot and several chemicals have been used to control the disease but no any concrete solution has been found and no any correct chemicals have identified yet to control Pythium properly [125]. Recent trends favour the use of alternative substances derived from plant extracts to control pests [88, 186, 72]. Natural plant products are important sources of new agrochemicals for the control of plant diseases [76, 99]. Their use in controlling diseases is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment, as they do not leave any toxic residues and therefore can effectively replace synthetic fungicides [32]. These natural products or plant extracts can be exploited either as leads for chemical synthesis of new agrochemicals, or as commercial products in their own right, or as a source of inspiration to biochemists for the development of new bioassays capable of detecting other, structurally simpler compounds with the same mode of action. The use of plants may offer a new source of antimicrobial agents with significant activity [105, 36]. Plant preparations have been used for centuries in medicine and pest control. Farmers in India use neem leaves to protect their stored grain from insects. Herbs and spices, such as basil and clove, have been used by many cultures to protect food from spoilage, as both have antimicrobial properties [14, 93]. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. Dahanukar and his coworkers [39] have reviewed the research on plant based antifungal compounds as a scientific approach and innovative scientific tool from 1994-1998. Antifungal activity of plant extracts against a wide range of fungi has also been reported by many workers [86, 56, 183, 37, 2, 185, and 165].

Researchers have suggested that antimicrobial components of the plant extracts cross the cell membrane interacting with the enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces change in the cell and ultimately their death [118, 113]. Other researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable hence leaking of ions and other cell contents can then occur causing cell death [30]. Antimicrobial screening of plant extracts is usually done with crude alcohol or aqueous extracts prepared either by cold or hot extraction methods. Crude or alcohol extract of several plants have been screened for their possible antimicrobial activities against pathogenic virus, bacteria, fungi and protozoa [6, 5, 42, 107, 122, 167, 152, 150, 100, 130, 144].

Pretorius and his coworkers [127] tested crude extracts from thirty nine plant species for their antifungal potential against seven economically important plant pathogenic fungi. Asanga [1] reported the antifungal activity of crude methanolic extracts of six plant species against three economically important phytopathogenic fungi. The aqueous extract of Zygophyllum fabago and ethanolic extracts of Allium sativum, Azadirachta indica and Curcuma longa were shown to inhibit mycelial growth of P. aphanidermatum, in in vitro condition [40, 158]. Leaf extract of Zimmu was also reported to be effective against Pythium in in vivo condition [106]. Al-Rehma and his coworkers [8] reported that the extracts of T. vulgaris and Z. officinale was found to be effective in controlling tomato damping-off diseases and extracts may be an attractive alternative for the use of natural product to control tomato phytopathogenic fungi (Pythium and Fusarium species) avoiding chemical fungicide application. Vinayaka and coworkers [177] reported that aqueous extract of Usnea pictoides was found to exhibit inhibitory effect on Pythium aphanidermatum which was isolated from rotten ginger rhizome.

All the active principles present in plants are usually aromatic or saturated organic compounds so they get extracted in alcohol or methanol hence initial screening of plants for antimicrobial activities; begin with their crude aqueous or alcoholic extracts [37]. Some proteins and glucosides etc. are soluble in water hence antimicrobial assay of antimicrobial principle is usually done with aqueous, 50% alcohol or 100% alcohol extracts.
Rahman and other [130] and Hussin and other workers [70] reported the antifungal activity of methanolic, ethanolic and boiling water extracts of *Barringtonia* leaves, sticks and barks. Crude methanolic leaf extract of leaves of *Newbouldia* was screened against some bacteria and fungi by Usman and Osuji [173]. Crude aqueous pod extract of *Lecaniodiscus cupanioides* also showed potent antifungal activity [111]. Vinayaka and other [177] reported the biocontrol efficacy of *Usnea pictoides* against *Fusarium oxysporum* and *Pythium aphanidermatum* which cause rhizome rot disease of ginger. Amadioha and Obi [9]. Olufolaji [112] and Mitali and coworkers (102) reported that many plant extracts inhibit spore germination and mycelial growth of pathogenic fungi and found significant as pesticides. It has been reported by Sagar and coworkers [143], Haouala [62] and Suleiman and Emua [164] that many plants extract have antifungal activity against *Pythium*. Bahraminejad and collegue [17], Bahraminejad [16] also reported the antifungal activity of crude aqueous and methanolic extracts of 97 Iranian plants against *Pythium*. Abdolmaleki et al. (4) reported that crude extracts of some plants such as *Zataria multiflora*, *Pinus halepensis*, *Carum carvi* could effectively control *Pythium* drechsleri. Abdolmaleki and coworkers. [4] and Kim et al. [82] Reported antifungal activity of *Cinnamomum zelanicum* and *Xanthium strumarium* against *Pythium* drechsleri. Ramnathan et al. [134] reported inhibitory effect of spider lilly (Crinum asiaticum) against *Pythium aphanidermatum*. Bhatt [27] recorded 94.4 (% inhibition of *Pythium aphanidermatum* with botanical extract of *Oxyspora paniculata*. Vanker and Patel [175] demonstrated the efficacy of leaf extracts of *Lawsonia inermis* and *Emblica officinalis* against damping off of *Pythium* species. Uma et al. [171] reported that *A. zapota A. squamosa*, *C. papaya*, *P. granatum*, *V. vinifera* and *C. colocynthis* showed antifungal activity against *Pythium capsici*, and *A. zapota*, *T. indica*, *C. papaya*, *P. granatum* *V. vinifera*, *C. colocynthis* showed antifungal activity against *P. aphanidermatum*. Parveen and Sharma [119] also reported the antifungal activity of crude aqueous, 50% hydroalcoholic and alcoholic leaf extracts of *Azadiracta indica*, *Aegle marmelos*, *Cassia fistula*, *Clitoria ternatea*, *Delonix regia*, *Eucalyptus globules*, *Jaccaranda mimosifolia*, *Justicia gendarusa*, *Moringa olifera*, *Murraya koenigii*, *Nigella sativa*, *Pongamia pinnata*, *Polyalthia longifolia*, *Tecomella undulate* and *Terminalia arjuna* against the *Pythium aphanidermatum* and *Pythium myriotylum*.

Initial antimicrobial screening with crude extract is followed by screening of extracts prepared in various organic solvents. Goel et al. [52] reported the antifungal activity of hexane, ethyl acetate and methanol extracts of *Parmelia reticulata* against *R. solani*, *F. udum* and *P. aphanidermatum*. Chapagain et al. [34] reported antifungal potential of saponin rich-extracts from *Balantias egypitaca* fruit mesocarp, *Quillja saponaria* bark and *Yucca schidigera* against common phytopathogenic fungi (*Pythium ultimum*, *Fusarium oxysporum*, *Alternaria solani*, *Colletotrichum cucodes* and *Verticillium dahliae*). Haouala et al. [62] and Suleiman and Emua [163] reported that an aqueous extract of *Fenugreek* and *Aloe* could inhibit mycelial growth of *P. aphanidermatum*. Sagar et al. [142] and Dileep et al., [47] showed the fungitoxic efficacy of *Ferula asafoetida* and *Azadirachta indica* plant extracts and ripe and unripe pericarp extracts of *Polyalthia longifolia* against *P. aphanidermatum*. Sohbat [160] reported anti-*Pythium* activity of methanolic extract of *Centaura sp.*, *Papaver dubium*, *C.beheen*, *C.depressa*, *Hypericum perforatum*, *C.iberica*, *Juglans regia*, *Quillja saponaria* and *Vanker and Patel*. [175] showed that acetone extract of *Moringa olifera*, *Murraya koengigii*, *Nigella sativa*, *Pongamia pinnata*, *Polyalthia longifolia*, *Tecomella undulate* and *Terminalia arjuna* against the *Pythium aphanidermatum* and *Pythium myriotylum*. Jeyasakthy et al [74] reported that methanol extract and ethylacetate extract of *A. indica* showed significant antifungal activity against *Pythium* sp. Vivek et al. [178] reported that acetone extracts of *Evernia prunastri*, *Hypogymnia physodes* and *Cladonia portentosa* were found to possess antifungal activity against *Pythium ultimum*. Plants have generated the interest of man for therapeutic values chiefly because of the presence of various secondary metabolites. Plants produce a wide variety of bioactive secondary metabolites which serve as plants defense mechanisms against pests. Some secondary metabolites give plants their odours (terpenoides), some are responsible for plant pigments (quinines and tannins) and others (e.g. some of terpenoids) are responsible for plant flavor. The antimicrobial properties of plant extracts is also because of presence of secondary metabolites such as alkaloids, phenols, flavonoids, terpenoids, essential oils etc. [64]. According to Cowan [37] the major groups of antimicrobial bioactive compounds are divided by into 5 main classes consisting: Terpenoids and essential oils; phenolics and polyphenols; alkaloids; polypeptides and mixtures (crude extract). Several workers have reported antimicrobial activity of secondary metabolites of plants [147, 129, 43, 34, 18, 24]. Ethanolic extract of 40 higher plants representing 23 families were studied by Begum et al. [22] for their antifungal activity against 6 phytopathogenic fungi (*Alternaria alternata*, *Curvularia lunata*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Botryodiplodia theobromae* and *Colletotrichum corchori*).
In another screening, antifungal effects of 66 medicinal plants belonging to 41 families were evaluated against *Pythium aphanidermatum*, the causal agent of chilli damping-off Muthukumar et al.,[106]. The Zimmu (Allium sativum L. × Allium cepa L.) leaf extract had the significant inhibitory effect against mycelial growth of *P. aphanidermatum* [188]. Wang *et al.* [179] reported a chitinase with antifungal activity isolated from *Phaseolus mungo* seeds. Chitinase is also reported to exert antifungal activity towards *Pythium aphanidermatum* besides other phytopathogenic fungi. Wang *et al.*, [180] reported that a novel lysozyme from *Phaseolus mungo* also exhibited antifungal activity toward *Botrytis cinerea*. Yazdani *et al* [186] reported the anti-*Pythium* activity of extracts of *Chaetomium aureum*, *C bostrychodes*, *C. cochlodes*, *C. cupreum*, *C. cupreum*, *Gliocladium catenulatum* and *G. catenulatum*. Hence according to the review regarding the control and management of *Pythium* diseases it is found that the plant extracts offer the best opportunity of success for disease biocontrol, and will help to maintain the quality as well as crop yield.

**CONCLUSION**

The current status of research suggests that there are indeed alternatives to replace the synthetic fungicides for management of this notorious soil as well as seed borne fungi: *Pythium*, which causes loss of multimillion dollars. However the farmers uses the common synthetic fungicides which leads into ill effects as well as many of the commonly used synthetic fungicides are unable to control *Pythium* species as it has got resistant against these synthetic fungicides. Hence there is need to replace the chemical fungicides by bio-fungicides, prepared from plant extracts and antagonistic microorganisms. Bio-fungicides will also be economical to the farmers and besides this the use of bio-fungicides will not leave any ill effect in the soil, water as well as in the environment. It is possible that by combining these approaches, (use of plant extracts, antagonistic micro-organisms, organic manure) an economically viable alternative for crop production system can be developed. So the use of bio-fungicides proved to be economic alternative that can be implemented at the farm level. For the effective production of crops, formulation protocols as well as its using methods should be provided to the farmers. Formulation must have adequate shelf life, stability, and titer. Before any formulated product is marketed, it must first be thoroughly tested by growers, whose comments, critiques, and suggestions for improvement, must consider.

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