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THE IMPACT CONTROLLING OF THE INCREASING PLANT PATHOGENS VIRULENCE TO PREVENTS ENVIRONMENTAL DEGRADATION

The Case of Leaf blight disease attacks on *Pinus merkusii* In Java - Indonesia

Sutarman
Faculty of Agriculture, Universitas Muhammadiyah Sidoarjo
Email: sutarman@umsida.ac.id

Abstract: Java has 6.8% of land area and inhabited by 57.5% population of Indonesia are mountainous with high rainfall and overgrown by pine (*Pinus merkusii*) forests that have a role on regional hydrology. The impact of global climate change had changed the existences and the behavior of the component of forest ecosystem. It was showed through the increasing of virulence of plant pathogen including *Pestalotia theae* which caused the pine leaf blight. This pathogen showed the high virulence on pine seedlings in the whole Java. The threat was higher since the crown stands become the inoculums sources which the infection did not caused the tree death. The adaptation of pathogen to climate change is suspected become the most important part in co-evolution of pines-pathogen. The attack of leaf blight pathogen which's destroys seeds from pine seedlings center in Central Java (1997-1999), West Java (2000) and East Java (2010-2015). The symptoms of infection on most of the crown of tree in forest become the indicators of the threat for pine forest existences. Some of the important strategies which needed to control the effect from the increasing of pathogen virulence are: (i) avoid using materials infested with the pathogen propagules as a growing medium of seedlings, (ii) utilizing the effective fungi from forest ground as controlling agent for pine pathogen and others which often intercropping on agroforestry system, and (iii) improving the method of tissue culture to create the high quality seed and avoid the seed using as pathogen vector.

Key words: Controlling strategy, Java, pathogen virulence, pines

INTRODUCTION

Indonesia has the second biggest tropical forest in the world after Brazil. Hence, Indonesia forest is most important part of world's lungs. On the other hand, forest becomes germplasm resources that can be utilize for food resources development and diversification and also provides various biological control organisms.

Another function of forest is as region hydrology controller. Despite that, various vegetation in forest such as small plants and trees rules on ground water regulation. *Pinus merkusii* is a species from Aceh, Kerinci, and North Tapanuli (North Sumatera). It is well developed in Java and growing naturally in Toraja Land (South Sulawesi) [37]. It has many role like fulfillment industry requirement (sources), give benefit to society economically, and as a water regulator mainly in high rainfall area. Hence, pine is suitable and growing well in area which has high rainfall 1,200-3,000 per year with altitude 200-2,000 m asl. [26]. Therefore, pines forests in high rainfall area become a water depositor and supplier for slopes and plains agricultural land through many springs in upstream.

Java as one of Indonesia islands which has pines forest with area about 14,219,000 Ha or 7% of Indonesia's land area. Java has a much heavy burden to be able to provide optimal support for human life and other biotic component of

ecosystems. In other hand, the area of Nation forest in Java is about 2,025,992.73 Ha or 21.3 % of land area [16]. Meanwhile, the area of pines forests which have role as water regulator is 476,126 Ha [44].

Population pressure on environment in order to fulfill life requirement is stronger, consequently, in the future this population have potential to face land degradation effects, such as water crisis, either clear water for consumption or water for production activities (e.g. irrigation, power plants, and some industries). Meanwhile, the global warming effect had already influence world climate change. And it impacts on degradation of forest function performance. As a result, threats to the carrying capacity of forests to meet the needs of living things in it will get stronger. Each ecosystem component changes will affect behavior change among biotic components including a wide range of disease-causing pathogens forest plants that will undoubtedly affect the guarantee system resources required for the fulfillment of human life.

One interesting case is the change in *Pestalotia thea* fungi virulence which was originally known as the plant pathogen minor. But, during the final decade of the 20th century the suspicion of fungi genus *Pestalotia* as one of the causes of diseases that endanger pine seedlings procurement system in the nursery was began to emerge. The results of observations conducted by researchers in the field of Microbiology Forest, Forestry Research-Ministry of Forestry of Indonesia (1998-1999) shows that *Pestalotia sp* and *Fusarium sp* cause disease symptoms of seedling dry leaves in the nursery center of Perum Perhutani Unit 1 Central Java in Majenang. The results of the study in 2002-2003 [46] showed *Pestalotia theae* had already cause seedling blight with the level of heavy attacks in the center of the nursery Perhutani Unit 3 West Java Cianjur Pongpoklandak. While, based on research conducted in 2014-2015 the same fungi causing severe damage in all nurseries in East Java. Overall this pathogen attack may threaten forest regeneration in whole pine plantation areas in Java.

On the other hand, various effective microbes can isolated from forest floor, and they can be used for plant protection, especially from cosmopolite type like *Trichoderma* fungi which known produce secondary metabolite [21, 59]. This substance can be used as controller for various crops diseases [24, 25, 31, 64]. It also can induct plant endurance on pathogen [2, 7, 8], and produce interaction between plant, *Trichoderma* and pathogen [60] which favorable for cultivation.

These fungi can also produce plant hormones [20] and nutrients to promote plant growth and yields [9, 19, 22, 23]. *Pseudomonas fluorescens*, an effective bacterium which help plants to grow [43]. It easily obtained in forest areas. Forest soil has high organic matter relatively; a rhizosphere condition which ensure the increasing of fungi arbuscular mycorrhizal diversity and soil biological activity [3, 4, 14] as well as the abundance of ectomycorrhizal partner symbiosis with pine roots such as *Scleroderma clumnare* and *Pisolithus arrhizus* [1, 45, 63] that contribute to forest carbon cycle [6, 23].

Changes in one type of microorganism behavior, as demonstrated by increased virulence, may be used as an indicator of changes in the other ecosystem component characteristics, both biotic and a-biotic. Within parasites and plant interaction, pathogens can become a selective force and responsible on evolution which occur between plant and pathogen [5].

This paper aims to show the changes of forest ecosystem component behavior which's indicated through the virulence increasing of leaf blight pine pathogen case and various ways to overcome the impacts.

PINE LEAF BLIHT AND PATHOGEN VIRULENCE

Pine seedlings leaf blight is one of the new disease was known in the 1990s discovered by researchers from the Forestry Research and Development-Department of Forestry. Very little publicity about this Indonesian's typical pine disease; while the crop development in Java is only done in the area managed by Perum Perhutani, a company under the responsibility of the Ministry of Forestry.

Fungi Pathogenic and Disease Symtomps. Isolation committed against leaf blight symptoms either from nursery or stands are always found a fungi *Pestalotia sp.* and *Fusarium sp. Fusarium sp.* known as one of the pathogens that cause damping off on pine seedlings. From the result of Koch's postulates which have been done [46] showed that from the two candidates fungi which cause disease with leaf blight symptoms, *Fusarium sp.* do not cause the disease but *Pestalotia theae*.

These are characteristics of pathogen fungi, as follows [48]: (i) morphology, these fungi have 5-celled spore with three dark central cell and two transparent ends cell, have size about 24.35 µm x 7.23 µm (Figure 1) [52], (ii) the initial growth process at room temperature, is: starting with the advent of germ tube size 2.88 µm (± 0.59 m) from the fourth cell or two cells after about 4 hours pedicel at first looked more bubbles and seem to be more transparent, tube sprouts to a size 5.95 µm (± 1.36 m) on a 5 hour incubation mass, and become hyphae yarn with size about 56.11 µm (± 21.66 m) after 12 JMI and have branching; the overall pattern of growth followed this regression model $Y = 59{,}24\text{-}18.16X + 1{,}55X^2$ where Y is the length of the tube sprouts, and X is an incubation period (for 4-12 hours) [51].

Figure 1. *Pestalotia theae* Sawada: conidiospore celled five (left) colony on PDA (right) [52]

Generally, attack mechanism starts from a stoma at the tip of the leaf that will penetrate cell wall with cellulolytic enzymes assistance. At beginning of leaf infection starts from top of cotyledon which on that time pine sprouts has already 3-5 weeks old after germination with a gradual progression of symptoms (Table 1) to death when seedlings over 6 months and we often found severe attacks symptoms on stands (Figure 2). Meskipun gejala mulai tampak sejak umur 2-3 bulan, tapi sesungguhnya infeksi sudah terjadi pada kotiledon bibit berumur 1 bulan.

Table 1. The development of pine leaf blight diseases in Pongpoklandak seedbed, Cianjur, (West Java) [48]

Seedling age	The Attack symptoms
2,5 – 4 month	The light attack start at the top of cotyledons, sometimes in the middle or base, the symptoms can be seen when the seedling in 2 – 5 month; after 3 month a single small leaf would be infected at the top of leaf part; the faster infected cotyledons would be yellowing, then the yellow brown color spread to base, and after that it would be dead; the middle part of infected leaf or the dead part have grey color, meanwhile the part that closer to infected part or closer to healthy part have yellow brown color.
4 – 6 month	Most of small/single leaf was attacked, older more dead leaf; double (needle) leaf was attacked since 4 month, older more leaf that attacked.; on one leaf, the infection could spread to base; the infected single leaf tends to be faster in yellowing; there were so many cotyledons fallen, even the seedling was 6 month old, there were no cotyledons could be seen (early fall). The characteristic of infection and death developments are relatively same not only on double (needle) leaf but also on single leaf or cotyledons. The base or middle part of leaf which's infected and undergo tissue death, will caused the top of leaf yellowing and death.
Over 6 month	Most of the needle leaf were attacked, the infection development became varies; single leaf relatively few (because there were many fallen leaf, the death fasten by infection). It was nearly 30% of seeds had 2 (in 0 – 4 scale) disease score which's in about next 1 – 3 month would be in danger of dying or the minimum effect was it unfit taken to the fields.

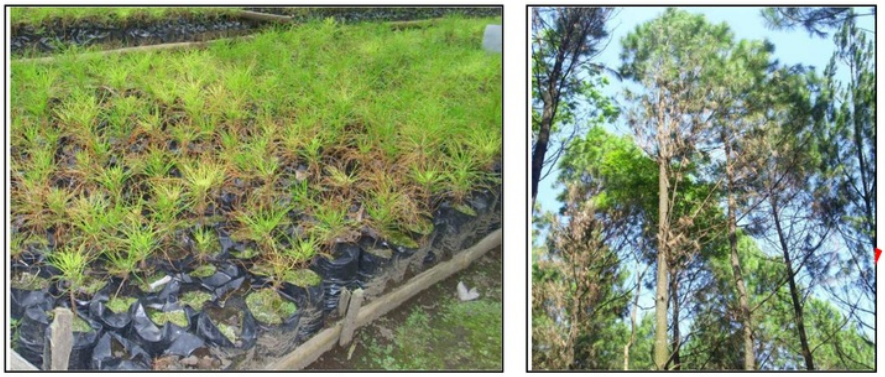

Figure 2. Leaf blight attack symptoms on seedlings (left) and on stands (right) (array sign) (Author Collection, 2014)

Pathogen Virulence and Environment. In their interaction with the pathogen, plant develops an ability to resist attack through response which represent on peroxide activities and polyphenoloxyd increasing. However, the activity of both defense enzymes are different on the header component, seedlings and plant health conditions. At the time of overspin, activity polifenoloksidase and peroxidase of

cotyledons respectively 0.00290 units of enzyme activity per gram wet weight (UEA/gww) and 1.1600 (UEA/gww) without showing symptoms of illness, but when the seedling age of 1 month the activity of these enzymes decreases in the amount of 0.00123 and 0.7300 (UAE/gww) on cotyledons healthy and 0.00109 (UEA/gww) and 0.3267 (UEA/gww) on cotyledons illness (infection) [51]. It shows that the decrease in the activity of polyphenoloxidase and peroxsidase will make the leaves more susceptible to pathogens.

The attack intensity which presented on disease index turned out to be strongly influenced by environment. Based the regression equation of some established model, it seems that the observations every two weeks with no lag time on all environment component model is a model that can be trusted to predict the influence of environmental component on plant disease index development. The best regression equation of *P. merkusii* seedling's leaf blight disease index in Pongpoklandak Seedbed, Cianjur is $Y = 91{,}9 - 1{,}59X_1 - 13{,}1X_2 + 5{,}96X_3 + 0{,}437X_4 + 0{,}0802X_5 - 0{,}749X_6 - 1{,}02X_7$ (R^2 = 83,2) [50]. It showed that only the average of daily rainfall (mm)(X4) (Pr>F = 0,007), the average of daily sun radiation (%)(X5) (Pr>F = 0,007), and the average of daily air relative humidity (%) (X6) (Pr>F = 0,009) have the most influence on epidemiology pine leaf blight growth, the other hand, the number of conidiospores that was caught from air per cm^2 trapped glass (X1) (Pr>F = 0,849), the average of daily wind velocity at height 0,5 m (km/hour) (X2) (Pr>F = 0,06), the average of daily wind velocity at height 2,0 m (km/hour) (X3) (Pr>F = 0,084), and the average of daily air temperature (°C)(X7) (Pr>F = 0,096) have not influence on the epidemiology [50].

In line with the epidemiological models of pine seedling blight turns the temperature and humidity at the place with the different altitude and the different environmental conditions influence the differences in disease index development. In Cijambu (Sumedang) with a height of about 1,000 m asl turned out to disease progression is slow and can be considered harmless, in contrast to Gunung Batu (Bogor) 450 m asl, especially in Pongpoklandak (Cianjur) 250 m asl at first observation (3 weeks after inoculation) disease index has reached 23.74 with 3 months old seedling [50]. Therefore seedlings which grown in Cianjur Pongpoklandak relatively threatened death when seedlings over 6 months.

Within a decade later, results of research conducted in East Java are not consistent with results of research in West Java. As shown in Table 3, same height didn't show the same disease index relatively. In addition to the air humidity daily averages, rainfall, average daily temperature, and other environmental factors, there is influence of intrinsic pathogenic fungi are realized in the form of different virulence between isolates in West Java and East Java. Another possibility is a change in the virulence level during a period of more than 10 years in response to climate change that occurred during this time.

Other facts show that early age infected seedlings did not affect the disease index growth. Index growth of leaf blight disease among nursery in East Java following this regression model: $Y = 9.848 + 1.573\ X_1 - 0.207X_2$, where X_1 is the time of observation, X_2 is the early age of seeds, with R2 = 0.941; meanwhile time observations of extra time from initial observation showed significant effect (t stat 35.839, P = 0.000) [52]. This shows that when infection has occurred, then in line with increasing time, infection will develop and disease symptoms intensity will increase.

Tabel 3. The mean disease index seedling of 2014 production year at various *P. merkusii* nursery in East Java with geoecological representation and coverage administrative area it serves [52]

Forest Management Unit (FMU) and it's coverage geoecological representation	Location of obervation (section of coverage administrative area of FMU)	Altitude (m asl.)	mean of disease index (0-100)
Malang (Southern Welirang -Arjuno-Anjasmoro mountains)	Pujon Selatan	1,000-1,200	37.2
	Wagir	900-1,000	50.3
Pasuruan (Welirang -Arjuno-Anjasmoro mountains)	Celaket	1,000-1,100	27.7
Bondowoso (Ijen mountain and its surroundings)	Wringin Tapung	300-350	31.9
Jember (Iyang mountain and its surroundings)	Garahan	500-600	30.0

Pine leaf blight pathogen is also potentially dangerous because it can survive on weed plants to infect leaf tissue showed symptoms until severe attack even though no cause of death. Cross inoculation test results among *Paspalum conyugatum* and *Ageratum conyzoides* (weeds), *P. merkusii* stands and *P. merkusii* seedlings as host to pine seedlings shows that the source of inoculum from the leaves of trees and saplings *P. merkusii* and dominant weeds in the nursery of pine turns infective against pine seedlings and saplings of both types of weeds healthy [49].

Analysis of damage to seedlings on center of production. The cases of pine leaf blight attacks that occurred in Central Java in 1997-1999 has resulted in severe dredged next to one pine seedlings location moved to another location with a height of 600 m asl than previously in the altitude of 200 m asl in Majenang Central Java. The attack occurred on a massive scale of this disease in the nursery center Perum Perhutani West Java Regional Division at 1998-2002 with damage seedlings up to 85% where from about 1.815 million seedlings only about 200 thousand seedlings that can be sent in the field with disease attack rate pertained average being [46]. For in the working area of Perum Perhutani East Java Regional Division, based on author's observations in 2014-2015, it is known that damage to seedlings in nursery forced operators in field had to do seedling replanting die seedlings up to 50% of the total population of planned seedlings. There is no publication of damage, making a loss potential of these diseases is getting less attention from public and stakeholders.

Coevolution of Plant-Pathogen. Case of pine leaf blight disease attack in West Java 1999-2002 indicates the behavior change of at least one type of pathogen. Pathogens commonly attacks tea and do not harm crop production. Before the 1990s, we didn't found plant interference case especially in nursery due to late blight pathogen attack, except the damping off attack. In the case of late blight pathogen attacks on trees seem to have occurred co-evolution without a winner as

stated [32], in contrast to the seed that seemed, parasite evolution is faster than the host, although this requires deep research. Pathogen becomes virulent on pine seedlings. With sprouts tube size 2.88 µm (± 0.59 m), then the fungi easily enter the stomatal size is much larger (Table 4). However, to break the cell wall is certainly not easy. Therefore, fungi prepares chemical weapons, i.e. enzyme glucose and poligalacturonase.

Table 4. The stomata opening size from cotyledons, first and needle leaf of *P. merkusii* seedlings aged 2-3 months after weaning [51]

Crown component	Length of Stomata Opening	Width of Stomata Opening
Cotyledon	18,81-24,59 µm	7,10- 9,52 µm
First leaf	24,93-27,65 µm	8,34-12,02 µm
Needle leaf	22,53-25,09 µm	7,15- 9,71 µm

Hydrolytic enzyme activity in the medium, which is overgrown *P. theae* shows the average is higher than the control. Glucose and poligalakturonase activity resulting from media CMS + extract of pine overgrown *P. theae* amounted to 0.6466 ± 0.0196 (mg/l) and 1.5209 ± 0.4238 (µmol/g), whereas only from media CMS + pine extract (control) respectively 0.5668 ± 0.1565 (mg/l) and 1.3834 ± 0.3690 (µmol/g) [51]. Sign or form that represents the interaction between the pine trees and fungal pathogens changes may be more modest than the co-evolution which indicated by leaf morphological variability of woody plants species in Northeastern Mexico as a form of adaptation and coexistence between species due to differences in edaphic and climatic conditions [36]. At the interactions of fungi *P. theae* and pine plantations the indicator virulence increasing shown by infection or symptoms intensity which are relatively higher on stands level in forest, also the attack symptoms intensity and seedling death percentage in nursery is increasing.

Environmental Degradation Hazard. Environmental degradation shows decreasing in environment carrying capacity for organism survival in it. Deforestation and degradation are the two main causes of global warming that contributing 15% of greenhouse gas emissions, remember forests as carbon sinks which mostly emitted by industry and transportation to the atmosphere [62]. So far, forests rehabilitation and development efforts were oriented on Reducing Emissions from Deforestation and forest Degradation (REDD) plus the role of conservation, sustainable management of forests and enhancement of forest carbon stocks [13] through various program activities. Nevertheless the deepening of research, observation, and study on parasites behavior change impact for plant disease and co-evolution that embodied in sustainability and preservation of pine plantations has not been revealed and considered important yet.

Pine forest stands as forest plantations for wood and sap production interest, require regeneration or replacement of dead plants due to massive attack (Figure 2). Disruption in the supply of seeds in nursery will threaten the availability of young plants in order to replant after harvesting. Replanting vegetation failure will grow different vegetation that will reduce land productivity.

DISCUSSION

Plantation forest damage can be inhibited and prevented by implementing several strategies aimed at preserving the functions of the region and optimizing the use of land for food security purposes.

Food security programs in Indonesia mandated the need for intensification and extension of food crops. That means there should be efforts to increase the use of natural resources, including land use production forest known as agroforestry. Implementation of agricultural commodity crops it is possible to optimize the productivity of the land. This effort is not easy to do given the many challenges that must be faced, among which are a potential threat against many pathogens cause plant diseases such as: *Phytopthora infestans* causes potato blight, *Fusarium* sp. causes wilt in potatoes [17, 27, 39, 54, 58, 59, 60, 61], and pine seedlings damping off, *Rhizoctonia* sp cause damping off the pine [1, 64], and *P. theae* causes leaf blight of pine [46, 47]. All these pathogens are always found in the pine forest land and so potentially damaging pine seedlings and has a relatively broad host range. Therefore, in the future these pathogens will become a serious threat to the effort of applying agroforestry system. Development opportunities agricultural crops such as corn, soybeans, potatoes, and a variety of vegetable crops can be ideally utilized as a form of agroforestry in the pine forest. In addition to the selection of agricultural plant species suitability age and phase in the growth of pine trees need to be prepared strategies to control some types of harmful pathogens as the cause damping off, root rot and leaf blight.

The use of pesticides to control disease in agroforestry systems have always relied on the application of pesticides is certainly risky poison the soil and agricultural products. The development trend of biotechnology strengthened for the creation of an alternative application of chemical pesticides [19]. One prospect that needs attention is the use of fungi effective for the purpose of providing: (i) the biocontrol agents that act as disease control biological [3, 22, 24, 38, 43, 58], (ii) an agent biofertilizer or as agent provider of nutrients for plants through the decomposition of organic materials [53, 57], (iii) a source of substances plant growth regulator [12], and (iv) biological agents, especially mycorrhizae fungi to help plants cope with environmental stress that often occur such as drought and drought and lack of certain nutrients [18, 28, 33, 34, 35, 41, 55].

Some *Trichoderma* isolates (Table 5) are intrinsically turned out to have a good ability to protect crops against pathogens, and be able to increase the availability of nutrients which produced from organic materials decomposition.

Aspergillus fungi were also found on the forest floor and could potentially be used as a biocontrol agent and biofertilizer, given the ability of fungi in the produce hydrolytic enzymes and amylolytic potential [29, 56]

It has long been in the making pine seedlings in the nursery always use top soil under stands of pine in order to want to take advantage of potential ectomycorrhizal fungi such as *scleroderma clumnare* and *Pisolithus arrhizus* [62]. Despite numerous attempts to change the habits of nursery operator has been carried out by competent institutions such as the Research and Development Agency Department of Forestry, Bogor Agricultural University, and other institutions related to providing an alternative production technology package of pine seedlings without top soil and rely on mycorrhizal isolates that have been tested; but good habits that have started nurtured back to the initial conditions. It is necessary for solving efforts in the form of technology packages that are easy to prepare and easy to apply.

Table 5. The results of the analysis of organic materials of planting medium which inoculated by *Trichoderma* isolates selected at 4 months of incubation [53]

No.	*Trichoderma* isolate	Altitude (m asl.)	C organic (%)	Organic matter (%)	C/N ratio
1	Jelbuk	310	5.841	10.102	9.898
2	Grujugan	350	5.428	9.405	9.362
3	Jatijejer 2	400	7.269	12.570	10.851
4	Jatirejo	440	6.797	11.762	9.714
5	Kalibaru	600	5.859	10.142	9.932
6	Pronojiwo	780	5.868	10.160	9.783
7	Prigen	900	5.973	10.334	9.045
8	Celaket	1,040	5.682	9.832	9.627
9	Pujon	1,200	6.402	11.070	9.697
10	Kontrol (without isolate)		8,250	14.270	16,113

*) The importance value is showing the controlling power of *Trichoderma* against pathogens

Pine seedling production is only done through seeding so far are at risk of infested seed-borne pathogens since in the field, greatly depending on the season, and limited production period. Death of seedlings or saplings in the nursery and in the field due to disease are common. It is necessary for reproduction that does not depend on time, efficient as it can be produced in large quantities in a relatively short period of time, and conserve plant species from extinction is through culture techniques in vitro [30]. In consideration of the efficiency and health of seedlings, tissue culture techniques can also be applied to produce plant seeds of pine-based agroforestry components mainly potatoes [61]

FUTURE RESEARCH AND DEVELOPMENT

Research on effective microbe not only the types of fungi but also the types of bacteria that are as biocontrol agents, decomposers, suppliers of nutrients for the plants need to be done in depth. In addition to research on effective microbe formulation that helps improve plant resistance to environmental stress (e.i. drought and shade), also need to do research on conformity with altitude differences represented by humidity, temperature, precipitation, wind, leaves, and type of soil. Minimize the use of seed potato pathogens with the results of in vitro culture and has been associated with mycoryzhae, as part of agroforestry systems based pine, is a challenging research [61]. Proving the role of mycorrhiza [10, 11, 41, 42, 53] together with denitrifying bacteria in the supply of N for plants [18] needs to be done in order to optimize the land to the success of agroforestry systems in the future. Various of effective microbes need to be tested the suitability of inter-species and the effect on pathogens which in turn encourages the testing of formulations of the superior types of fungi and bacteria of the forest floor. *Trichoderma harzianum* combined with the application of certain pesticides can increase crop yield and effective control of damping off [15], as well as on the use of P. fluorescence [40].

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