Entomopathogenic fungi as biological agents in forest plant pest control: A systematic review

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Abstract. Entomopathogenic fungi have been seen application in pest control due to being more friendly to the environment compared to synthetic insecticides. This research aims to determine the potentials of entomopathogenic fungi by identifying research reports regarding the effectiveness of fungi as biological agents for controlling pest insect attacks on forest plants. This research used a systematic review to enable extended analysis. The systematic review was performed on secondary data obtained from 51 scientific articles concerning tests of entomopathogenic fungi on forest plants pest insects. The research publications originated from multiple continents and a surge in the number of publications took place in 2020. Curculionids were the most frequently found pest insects with 14 species encountered. The most frequently found and tested stadium of insects on the field was the adult stage used in 39 researches. The most dominant fungi used in the researches were ascomycetes with 31 species, ten species among them originating from the Cordycipitaceae family. The most used application methods were direct spraying and immersion, each with a 33% percentage of usage.

1. Introduction
One of the obstacles in the global forest industry is plant pests, particularly in Industrial Forest Plantations (HTI). Production forests furthermore are mostly monocultures vulnerable to pest attacks and pest population explosion. Pests can cause loss and value degradation of forest products which calls for efforts to control and lower the population of pests to an equilibrium.

Post-World War II insect pest control efforts mainly used Dichlorodiphenyltrichloroethane (DDT) mixtures, while the most frequently used European pest control methods were pre-planting immersion of seedlings in or post-planting spraying with alpha-cypermethrin and cypermethrin synthetic pesticides. Both of which are prohibited by the Forest Stewardship Council due to their dangerous effects and FSC-certified companies are expected to find alternatives [1]. Application of synthetic chemical insecticides on forests is not recommended considering the potential of increasing the resistance of target pests to control efforts, eliminating natural pest enemies or beneficial insects, decreasing biodiversity, spreading poisonous substances to humans, and polluting the environment [2]. Such application may also cause long-term biomagnification of toxic compounds in the food chain which endangers the lives of virtually every living being, particularly humans. The aforementioned negative effects necessitate sustainable integrated pest control strategies based on ecological principles and government-issued obligation for efforts based on Integrated Pest Control or Management (IPM).

Usage of natural enemies is a principal part of IPM carried out by utilizing the pathogenic traits of certain natural agents. Pathogenic fungi are a type of biological agent applicable as biopesticides owing to their capability of infecting certain host insects through cuticular penetration. These fungi also have high spore spreading capability on the field with high survival rates, for example, the conidia of...
Metarhizium anisopliae that can last up to 3.5 years underground [3]. Fungi additionally have the characteristic of mostly attacking specific targets thus reducing the side effects and risks on non-target organisms such as beneficial insects to a great extent [4]. Entomopathogenic fungi have been developed as alternatives to synthetic chemical pesticides or combined with it to reduce overall synthetic chemical impact. Usage of entomopathogenic fungi as bioinsecticides are deemed more friendly to the environment based on the aforementioned characteristics and are more biologically persistent compared to the application of synthetic chemical insecticides. Researches regarding entomopathogenic fungi as biological pest insect controllers are often based on mortality and available isolate virulence analysis, however, analysis on the range of fungi, pest insects, and pest afflicted plants in forestry studies are also needed to provide information about the progress of researches and broaden the scope of characteristics needing evaluation for large scale developments.

2. Methods
2.1. Tools and Materials
Tools used in this research among others are stationaries and personal computers with internet connection and Microsoft Office applications Word and Excel. The materials are secondary data from officially publicized scientific articles on accredited national scientific journals and reputable international scientific journals indexed in Scopus. This research analyzed 51 articles reporting the effectiveness of fungi as biological agents for controlling pest insect attacks on forest plants.

2.2. Data Collection Procedures
2.2.1. Identification of Keywords. The systematic review starts with the identification of keywords used for article searching. The keywords were obtained from previous researches related to the topic of entomopathogenic fungi as biological agents for forest plant pest insect control. Keyword combinations include “Fungal Pathogens” or “Entomopathogenic Fungi” or “Pathogenicity” and “Insects Pests” or “Forest Pests”, and Indonesian keyword combinations such as “Bioinsektisida” or “Insektisida Alami” or “Fungi” or “Entomopatogen” or “Cendawan Entomopatogen” and “Serangga Hama” or “Hama Hutan”.

2.2.2. The search of Articles Related to the Keywords. National and international scientific articles were searched on official accredited sites including but not limited to Science Direct, Proquest, Gale Cengage, and FORDA.

2.2.3. Filtering Article Search Results. Filtering was carried out by choosing the articles fulfilling the qualifications of possessing the needed keywords and not being a review article.

2.2.4. Determination of Article Suitability. Article suitability refers to the possession of related information—that is entomopathogenic fungi as biological agents for forest plant pest insect control—within the accredited article.

2.2.5. Data Analysis. The last procedure of this systematic review was the analysis of the research report articles focusing on the effects of entomopathogenic fungi on forest plant pests insects. Variables used among others were the year of publication, the geographical distribution of researches, species of fungi, fungi application methods, species of pest insects, range of insect stadia, and species of forest plants. Analysis results show the development of researches on entomopathogenic fungi application in forestry.

3. Results and Discussion
3.1. Research Ranges
Development data of researches on entomopathogenic fungi as biopesticides in forestry is deduced from the sum of published articles within years as shown in Figure 1. A sum of 51 articles had been found published within 20 years (2002-2021), but there had been an absence of publication in the years 2004, 2005, and 2007. One of the causes for the absence was the insufficiency of information regarding entomopathogenic fungi. A variable sum of publications was seen from the rest of the years. The years 2002, 2003, and 2006 each has only one publication. The years 2009, 2011, 2012, 2014, and 2021 each yielded two articles. The years 2008, 2010, 2015, 2016, and 2017 each has three articles published. The years 2013 and 2019 yielded four articles and 2018 has the highest number of publications at six articles. These inclinations in publications are directly proportional to the rise of interest regarding forest pest control with entomopathogens.

![Figure 1](image)

**Figure 1.** Years of publications about applications of entomopathogenic fungi on forest plant pests

Insects used in each research regarding entomopathogenic fungi were collected from different cases of pest attacks in different countries. Cases from the articles are spread in Asia, Europe, the Americas, Africa, Australia, and the Eurasian regions. The United States (US) became the country with the highest number of publications (14 articles), while other parts of the Americas starting with Mexico and Canada each yielding two articles, and then Brazil and Chile yielded three and one articles, respectively. The Sum of European articles starts with the United Kingdom (UK), Poland, and Croatia each having one article published, and finishes with Ireland which published the most articles with three articles. Asian countries start with India and Indonesia which came up with one article from each country, followed by South Korea with two articles, and end with the People's Republic of China who published eight articles becoming the country with the second most articles published. The African countries were Kenya and Morocco each with one article published. Australia and New Zealand published 1 and 3 articles respectively. Turkey in the Eurasian region published four articles.

The US being the country with the highest number of articles shows a high interest in entomopathogenic fungi application as an alternative pest control method in the country. One of the causes for the interest was the US' national forest management program that used periodic forest monitoring activities which enables continuous and thorough re-evaluations on forest health [5]. Awareness about the health status of Indonesian forests in regards to sustainable forest management is relatively still low [6]. Forest health is a basis for the control over the rate of damages inflicted on forests, which aims to bring the rate to an acceptable level below a certain economic threshold. The concern over forest health becomes a reason for re-evaluating the support for research and development on pest control in Indonesia.
3.2. Range of Forest Plant Families

Figure 3 shows the various forest plant families afflicted with damage from pest attacks in different countries. Plants receive pest-inflicted damage from becoming shelters and sources of food for various pest insects.

The Pinaceae family received the most pest-inflicted damages. The family was represented by thirteen species, they were *Abies procera*, *Picea engelmannii*, *Picea sitchensis*, *Pinus contorta*, *Pinus halepensis*, *Pinus massoniana*, *Pinus radiata*, *Pinus sylvestris*, *Pinus tabuliformis*, *Pinus wallichiana*, *Pseudotsuga menziessii*, *Tsuga canadensis*, and *Tsuga caroliniana*. Pine stands frequently become targets for pest attacks due to monoculture practices in plantation industries. The low value of species diversity accompanied by high stand populations become the cause of pest population explosion which may inflict large-scale serious damages on the forest. Damaged pines show scraped bark, bent needles, withering branches, drooping shoots, and tree death. Pine stands are generally vulnerable to shoot miners that cause bent stems and larger branches which lower the product quality and quantity [7]. The ranges of other forest plants were Fagaceae with five species, Oleaceae, Myrtaceae, Sapindaceae, and Moraceae each with three species, and lastly Betulaceae, Proteaceae, and Salicaceae each with 2 species. All other families not mentioned only have one afflicted species.

The types of forest stands affect the microclimatic conditions such as temperatures, relative humidity, and C/N ratios, and also affect edaphic conditions such as soil texture, pH values, and organic material content. The aforementioned variables affect the abundance of fungi species. Research results show that the abundance of entomopathogenic fungi depends—among other variables—on a high amount of organic material and moderate pH value (around 8) on the growth media [8]. Root exudates also become sources of food and energy for fungi in the rhizosphere, therefore it is concluded that the type of tree stand affects the existence of entomopathogenic fungi. Development of fungi must be supported by forest condition variables including but not limited to geographical location, habitat type, soil types, and soil processing in forest plantation management, along with the range of available local fungi species able to survive in the plantation stands.
3.3. Range of Insect Species and Stadia

Results of identification on insect species reported to be the causes of damage on forests yielded 41 species distributed in 6 orders and their reported number of families, which were Hemiptera (seven families), Lepidoptera (six), Coleoptera (five), Isoptera (one), Diptera (one), and Thysanoptera (one). The number of species from each family is shown in Figure 4. The species found to be forest plant pests originate from the families Curculionidae (fourteen species), Cerambycidae (five species), Lasiocampidae (three species), and Coccinellidae (two species). There are also seventeen additional families each having only one pest species. Curculionids were found attacking forest plants from the families Pinaceae, Lauraceae, Fagaceae, Juglandaceae, Proteaceae, Euphorbiaceae, and Betulaceae.

Most forest plant pest insects are phytophagic species. Phytophagic species can be monophagous, oligophagous, or polyphagous. Monophagous types consume only one type of plant in a genus, oligophagous types consume only one type of plant in a plant family, and polyphagous types consume various plant species from various families [9]. The consumption behavior of each insect species must be monitored based on the aforementioned consumption type information. Polyphagous types may be the pest with the largest range of plant species targets. Coleopterans, Hemipterans, and Lepidopterans were reported to be attacking various Pinaceae species.

Fungi can infect host insects in various life stages be it egg, larval, pupal, or adult stage. Host specifications depend on the nutritional needs of the fungi and the defense mechanisms of each insect. Identification of stadia used in the researches which represents the field conditions yielded a comparison data of stadia used among researchers, as shown in Figure 5. The dominant stadium used in the researches in the adult stage (39 usages) mostly constituted by Coleopterans (28 usages). The larval stage was the second most used stage (fifteen usages) and is followed by the nymphal stage (eight usages), pupal stage (four usages), and egg stage (two usages). The adult stage has the most used due to being easier to be found in the field. A review of the research articles shows that each developmental stage of insects has different rates of vulnerability or resistance against fungal infection. Adult insects have a thin cuticular layer and intersegmental folds forming a microclimate with high humidity that supports easier germinating for conidia that cling to the insect, causing a higher death rate compared with other stadia [10]. Adult insects are also more active in consumption and ovipositing, resulting in a higher potential of contact with substrates containing spores of fungi and spreading it from the infected self to healthy insects [11].
Conidia given to larvae mostly only cling to the setae and do not touch the cuticles, and when the larvae move around the conidia may detach from the intersegmental membranes, which caused larvae to be deemed relatively not vulnerable to fungal infection [12]. Nymphal stage insects were also reported to be less vulnerable to fungal infection and have lower mortality rates. Nymphs can moult into the next instar in its development which detaches whatever spores may have clung. Body surface wax layers also inhibit clinging, germinating, and invasion of fungi. Thicker cuticles give additional inhibition of fungal penetration. Cuticles of insects especially the procuticles are polymer structures made of chitin polysaccharides. A thicker cuticular layer that has more proteins and chitin will cause invading fungi to produce more extracellular enzymes and use more time to degrade the cuticles. Pupae have thin cuticles but are not vulnerable to fungal infection due to being wrapped in a chrysalis covered with wax made of various components particularly long-chain alkanes, fatty acids, fatty alcohols, esters, cyclic alcohols, and cyclic acids. These various compounds protect the insect by inhibiting entomopathogen invasions [10].

Figure 4. Range of forest plant pest insects mentioned in the publications

Figure 5. Stadia of insects causing damage to forests, as used in the research articles
3.4. Range of Entomopathogenic Fungi Families

There are 34 fungi species distributed in twelve families used in pathogenicity tests on forest pest insect species in the research articles, as shown in Figure 6. The families Ophiocordycipitaceae, Nosematidae, Saccharomycetaceae, and Myriangiaceae each have one species of fungus. While the families Davidiellaceae, Trichocomaceae, Entomophthoraceae, and Hypocreaceae each have two fungi species. The families Nectriaceae, Clavicipitaceae, and Cordycipitaceae respectively have three, eight, and ten species of fungi. The fungi with the highest occurrence are the Ascomycota with 31 species, while other divisions being the Zygomycota, Entomophthoramycota, and Microsporidia each only has one species.

![Figure 6. Range of entomopathogenic fungi families used in the research articles](image)

Ascomycota is fungi with haploid mycelia, septa, and ascospore (sexual spores) produced in a sac called an ascus. The identified species that were found infecting insects are from the genera *Metarhizium*, *Hypocreia*, *Beauveria*, *Hirsutella*, *Isaria*, *Lecanicillium*, *Myriangium*, *Colletotrichum*, *Acremonium*, *Fusarium*, *Aschersonia*, *Aspergillus*, *Cladosporium*, *Candida*, *Cordyceps*, and *Paecilomyces*. The factors affecting the virulence of fungi on insects are germination and sporulation power, infection rates, conidium size, appressorium formation, propagule hydrophobicity, and production of cuticle-breaking enzymes or toxins against insects [13]. Toxins produced by entomopathogenic fungi have an important role in killing the host by breaking down the organic structures and then causing cellular dehydration preventing tissue regeneration. The mycotoxins produced from ascomycetes among others are beauvericins, beauverolides, bassianolides (from *Beauveria bassiana*, *Verticillium lecanii*, and *Paecilomyces* spp.), and destruxins (from *Metarhizium anisopliae*) [14]. Destruxin compounds caused immunomodulation or modification of immune system responses from insects, which raised the insect's vulnerability [15]. Ascomycetes *Beauveria bassiana*, *Lecanicillium lecanii*, *Metarhizium anisopliae*, and *Metarhizium flavoridus* in addition were proven to be able to produce lytic enzymes that break down cuticles, such as the enzymes proteinase, chitinase, and lipase that helps the entomopathogen in penetrating the cuticles [16].

3.5. Fungus Application Methods

The application of entomopathogens needs an appropriate method to optimize the infection process. Figure 7 shows the usage percentages of methods used in the reviewed publications.

The most used application method was spraying and immersion. Spraying was differentiated into direct spraying on insects (33% usage) and spraying on substrates (29% usage) such as tree bark, branches, filter paper, stumps, leaves, and twigs. The mentioned substrates were chosen due to being the places of insect activity thus having the potential of imparting spores to the insects. Immersion methods (33% usage) use a variety of immersion durations which are five, ten, twenty, thirty, and sixty seconds. Immersion is carried out by soaking the insect in a solution containing conidia or spore
formulae. The rest of the methods are carcass contact (2% usage) which is performed by letting a healthy insect have direct contact with a sporulating insect carcass, and injection (4% usage) to test the immune system response of insects. Entomopathogenic fungi modulated the immune system response of insects by modifying the abundance of protease inhibitors, detox enzymes, antimicrobial peptides, and cellulotic metabolism proteins—found primarily in *Hylobius abietis* larvae [17].

![Figure 7. Entomopathogenic fungi application methods used in the publications](image)

The immersion and spraying methods were by far the most used application methods but the percentages do not describe the effectiveness rates. Pathogens can enter the insect anatomy through two routes, the first is passive entry by way of accidental ingestion, and the second is active entry through natural orifices or direct penetration into cuticles. Application on substrates might make both entry routes available. Comparative research from [18] showed that dry conidia are more effective than wet conidia, causing a death rate of 100% within only 12 days. The higher death rate was caused by a lower surfactant content, which increased the adhesion of conidia to the host. Dry conidia additionally preserved infectious spores, increasing the death rate further [19, 20] also proved beetles that received the infection from walking on woody stems experienced more mycosis than beetles immersed in the fungal solution.

Application of dry conidia has a drawback of potential inactivation by UV exposure, removal of inoculum by raindrops or stem rainwater streams, and fluctuating relative humidity and temperature. These drawbacks necessitate a hydromulch formulation to increase water retention capacity, maximize conidial production and viability, and reduce the effect of external weather modifiers. Hydromulch is a wetter condition than the surrounding air after spraying or rain can experience evaporation reducing conidial production, and when the surrounding air is wetter condensation will soak the hydromulch with moisture and increase the conidial production again [21].

Biological agents in pest control generally work slower than synthetic chemicals. A combination of insecticides and entomopathogenic fungi may form additive, antagonistic, or synergic interactions. Such combinations mostly behave additively and can delay the pest insects' resistance expressions against insecticides. Combination applications have been implemented in a handful of researches, for example [22] have proven that combination with systemic insecticides (imidacloprid in particular) indirectly increased the pathogenicity and virulence of fungi. Insecticides absorbed by plant roots and translocated to the leaves increased the vulnerability of insects consuming the leaves, though not killing the target insects. Fungi when existing as endophytes are in a protected environment not exposed to abiotic nor biotic factors capable of limiting the effectiveness of fungi if the fungi were applied on leaves or the ground. The fungi’s existence in the cambium layer unfortunately may still be reduced in effectiveness by antifungal compounds produced by plant tissues, such as pinene and turpenol [23]. Some insecticides such as carbamate mixtures which behave as fungicides are also dangerous for fungal biocontrol agents. Researches have shown that an insecticide that might be effective against one insect species might perform less effectively or even weakly against others. Application of deadly
entomopathogenic fungi in conjunction with insecticides is hoped to be able to reduce the negative impacts and usage rates of synthetic chemical insecticides.

4. Conclusions and Recommendations

4.1. Conclusions

Results of the analysis on entomopathogenic fungi biological agents' effect on forest plants pest insects show that there has been a surge of research publications in the year 2020, proven by the number of publications that reached nine articles. Research has improved on multiple continents, with the US yielding 14 articles on the aforementioned topic. The insect family of Curculionidae has been found to be the family producing the most pest attacks with 14 species responsible according to the articles. The most frequently found and tested stadium of insects on the field was the adult stage used in 39 researches. The most dominant entomopathogenic fungi used in the researches was division Ascomycota with 31 species, 10 species among them originating from the family Cordycipitaceae. The most used application methods were direct spraying and immersion, each with 33% percentage of usage.

4.2. Recommendations

Most researches only test the effectiveness of entomopathogenic fungi in a lab condition without enough consideration of ecological limitations such as environmental factors. This fact made advanced research necessary in order to ascertain the effectiveness rates in various environmental conditions.

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