Entomopathogenic Fungi as an Alternative to Harmful Commercial Pesticides

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ABSTRACT

Since commercial chemical pesticides effectively eradicate pests that would otherwise destroy our crops, the agricultural industry relies on these pesticides to protect our food supply. As useful as these pesticides seem, many substances present in them pose a threat to our health. Consequently, biopesticides such as entomopathogenic fungi are being researched to reduce the use of these hazardous pesticides. Entomopathogenic fungi effectively attack pests without posing a risk to humans. This paper analyzes the adverse effects of commercial pesticides on human health and discusses the effectiveness of multiple species of entomopathogenic fungi in controlling pests.

Key Words: entomopathogenic, fungi, pesticide, cancer, biopesticide, mycopesticide

INTRODUCTION

Commercial pesticides are used widely in the agricultural industry and provide an extremely effective and efficient solution for deterring pests. They are regarded as essential to the agricultural industry as they prevent pests from attacking crops and jeopardizing our food supply.

As beneficial as pesticides may seem, they have numerous drawbacks since they are the source of major health concerns, and they are known to deteriorate the structure of DNA and cause illnesses such as cancer. Because of this, many biopesticides are recently being researched to help combat the use of commercial pesticides. One such biopesticide is mycopesticides, more commonly referred to as entomopathogenic fungi, which is a type of fungi that specifically targets insects. These are useful as they share the pest controlling abilities with commercial pesticides, but they lack the harmful effects that such chemical pesticides bring upon humans. As a result, numerous experiments have been conducted to test the effectiveness of these mycopesticides to potentially replace harmful pesticides used in the agricultural industry.

A better understanding of the health issues caused by pesticides and knowledge of eco-friendly pesticide alternatives will benefit the health of the population, especially those who live in agricultural communities. It would be useful to conduct extensive research analyzing the health concerns caused by pesticides and establish entomopathogenic fungi as a viable, eco-friendly alternative. Information is needed regarding the mechanisms that these insect pathogens utilize to eradicate pests, as well as the various genera of fungi that harbor entomopathogenic species. Therefore, this study provides this information and determines the effectiveness of mycopesticides by a series of experiments that introduce a variety of pests for the fungi to exterminate.

Because entomopathogenic fungi do not pose any threats to our health, they are much safer to use than commercial pesticides. As such, those who normally apply these harmful pesticides should instead use a species of entomopathogenic fungi to effectively remove pests from their plants while maintaining a healthy ecosystem.

REVIEW OF LITERATURE

While it may seem that the agricultural industry applies pesticides with extreme caution to prevent any contamination to surrounding wildlife, the trajectory of pesticides are not easy to control and can easily be introduced into the human body via ingestion of pesticide residues or inhalation of pesticide sprays. From here, the pesticides can cause a multitude of severe illnesses and may even lead to death.

Pesticides cause a variety of health concerns, one of them being the reduction in telomere length in human cells. Telomeres play an important role in our cells as they protect the ends of chromosomes from fusing together. As part of the natural aging process, the length of telomeres shorten after each cell division; after passing a certain length threshold, the cell may undergo cellular apoptosis, or programmed cell death. To prevent this from happening, an enzyme called telomerase reverse transcriptase helps to restore the length of telomeres. These processes ensure that telomeres are intact and effective for as long as possible. However, the increase of telomere shortening caused by pesticides may introduce an imbalance in this process, and telomeres may significantly shorten before their length can be restored. A study done by Hou and colleagues determined which pesticides pose a threat to telomere length. They examined the effect of 48 pesticides on telomere length in male individuals and found that seven pesticides drastically shortened telomere length. Moreover, those pesticides caused oxidative stress in cells, which has been found to severely damage and shorten telomeres. Additionally, pesticides can also result in decreased telomere length by causing inflammation. By triggering an inflammatory response, pesticides cause cells to exhibit increased proliferation, causing telomeres to shorten. This may cause severe cell damage and potentially cause cancer (Hou *et al*., 2013).

Pesticides have also been associated with a higher risk of developmental injury in human embryos. Chemical residue from pesticides were found at various concentrations, all of which were within the safe level for humans, in numerous body fluids associated with reproduction and fetal development. A study was conducted by Greenlee and colleagues to determine how exactly this translated to hindrances in embryo growth. They tested 13 chemicals and 6 mixtures at concentrations found in numerous agricultural pesticides and found that 12 of the 13 chemicals and 6 of the 6 mixtures resulted in developmental injury, most of which was embryos formed with fewer cells as a result of an increased rate of cell death in the embryos' early stages. This cellular loss may result in various forms of miscarriage or unhealthy pregnancy. Furthermore, these forms of pesticide-induced injury occur at concentrations lower than what is considered to be safe for human use. Because of this, it can be concluded that pesticide use translates to numerous potentially fatal developmental injuries in embryos, and decreased pesticide use will benefit the safety of embryos (Greenlee, *et al*., 2004).

Pesticide drift in the air is also a major cause of harmful pesticide exposure in humans, accounting for 37-68% of pesticide illnesses among agricultural workers in the U.S. Furthermore, 31% of pesticide illnesses in U.S. schools are caused by exposure to pesticide drift. To ascertain the health concerns linked to pesticide drift, Lee and colleagues conducted a study to identify and analyze 2,945 cases of illnesses associated with pesticide drift across 11 U.S. states from 1998 through 2006. Of these cases, individuals in close proximity to agricultural areas exhibited the highest amount of pesticide poisoning. Aerial pesticide applications (i.e. sprays) were responsible for 24% of cases, while fumigants originated from soil applicants were responsible for 45% of cases. Given this, a reduction in pesticide use will prevent such illnesses from affecting people and will therefore improve the health of agricultural communities (Lee *et al*., 2011).

Agricultural pesticide use has also been linked to greater rates of cancer in children. Children in agricultural communities have increased exposure to pesticides when compared to children in non-agricultural communities due to contact with contaminated air and dust, contaminated agricultural workers, and pesticide-treated crop fields. Consequently, they may also exhibit illnesses and other harmful effects caused by this exposure. A study done by Reynolds and colleagues analyzed the effects of pesticides on these children. They organized 7,143 childhood cancer cases from 1988-1994 into block groups according to population data, agricultural pesticide usage, and exposure to pesticides. From analyzing this data, they found that 1,233 block groups used more than 162 lb/[mi.sub.2] of pesticides each year, meaning that they were potentially at risk of developing cancer. Moreover, they found that an increase of pesticide use in fields has a direct correlation with pesticide concentrations in ambient air, leading to increased pesticide levels in children as they may inhale the residues. From this data, it can be concluded that an increase in pesticide use directly results in a higher risk of childhood cancer (Reynolds *et al*., 2002).

When regulations were imposed on the use of pesticides, poisoning incidents related to pesticides decreased. In developing countries, such as Sri Lanka, pesticides were used as a method of boosting the economy, as they were able to invest heavily in the agricultural sector without the fear of complications due to pests. As a result, these countries exhibited an extremely high rate of poisoning from these pesticides (specifically 15% in Sri Lanka), and without access to antidotes and proper intensive care, many casualties arose from these pesticides. To combat this, Sri Lanka imposed pesticide regulations in the 1990s to ban class I “extremely and highly hazardous” and class II “moderately hazardous” pesticides from commercial and personal use. After these regulations were enacted, poisoning deaths related to pesticides decreased from 83% to 77%, or an overall 7.22% decrease. Based on the aforementioned harm caused by pesticide exposure and the direct relationship between the decrease in pesticide usage and the decrease in poisoning-related deaths, it can be concluded that commercial pesticides are hazardous to public health (Roberts *et al*., 2003).

Due to the severe threat that pesticides pose to our health, more and more alternative biopesticides are being discussed. The intent of these biopesticides is to provide an effective pest control solution while maintaining the health of the environment and preventing the illnesses that commercial pesticides cause. One biopesticide that is extremely effective against agricultural pests is entomopathogenic fungi, or fungi that eradicates insects by attacking and infecting its host. It can infect its host via ingestion, wounds, or trachea. From here, the insect is killed by either starvation or toxin production. However, before the fungi can infect the insect, it first must penetrate the insect cuticle. After using physical or chemical processes to bypass the cuticle, the fungi can then grow directly into and steal nutrients from its host, effectively acting as a parasite as it kills the insect (Gul *et al*., 2014). At this point, to continue its parasitic behavior, the fungi must first evade the immune system of the insect. To do so, the fungi deactivates sensors in the insect that would normally obstruct the fungi and activates a pathway for the spores to travel throughout the insect's body. This enables the fungi to develop a pathway for nutrient drainage from the host and effectively evade host recognition. In order to deplete nutrients from its host, the fungi first releases adhesion proteins that bind the spores to the fungi's surroundings, including insect cuticles and plant surfaces (Wang *et al*., 2016).

After the entomopathogenic fungi successfully invades and parasitizes its insect host, it must then reproduce to spread to other insects. To do this, the fungi first colonizes and then produces spores inside the host. Then, it propels the spores out of the deceased insect to infect and invade other victims. To enhance the likelihood of encountering new hosts, the fungi produces large amounts of spores and utilizes wind, rain, and organisms such as invertebrates to help migrate the spores to more hosts. From there, the fungi can now spread throughout multiple hosts quickly and can eliminate a large population of insects (Gul *et al*., 2014).

Surprisingly, there are more than a thousand species of entomopathogenic fungi distributed across five phyla, most of which belong to the *Hypocreales* order. These species of entomopathogenic fungi infect a wide variety of hosts, including insects, mites, and spiders. Different genera of entomopathogenic fungi within the *Hypocreales* order are Metarhizium, Isaria, Beauveria, Cordyceps, Ophiocordyceps, Aschersonia, Lecancillium, and Tolypocladium (Wang *et al*., 2016). Within these genera, multiple species of fungi are currently being tested and used as mycopesticides against certain pests, including but not limited to *Metarhizium anisopliae*, *Beauveria bassiana*, *Cordyceps fumosorosea*, *Cordyceps javanica*, *Paecilomyces variotii*, *Hirsutella citriformis*, *Acrostalagmus aphidium*, *Fusarium culmorum*, *Lecanicillium lecanii*, *Cladosporium oxysporum*, *Capnodium citri*, and *Stemphylium* sp. (Ou *et al*., 2018). The following experiments test some of these species along with a few others, and they aim to establish the viability of entomopathogenic fungi.

Experiment 1

M. anisopliae and *C. fumosorosea* are both extremely well-studied entomopathogenic fungi that have been known for years to effectively control a variety of hosts. Recently, another species within the *Cordyceps* genus, *C. javanica*, has been discovered, and researchers believe it can potentially be entomopathogenic, much like other species in its genus. To determine whether this new strain can be as effective as *M. anisopliae* and *C. fumosorosea*, researchers set up an experiment to test all three fungi against *Diaphorina citri*, or the Asian citrus psyllid (Ou *et al*., 2018). They first isolated all three strains and individually sequenced them. Then, they grew *Murraya paniculata* plants and placed them in pots 30cm in diameter. The soil used to grow the plants was composed of 10% sand, 5% clay, and 85% peat. The colony of *D. citri* was collected and placed in equal amounts in each pot. They also used all five stages of *D. citri* during their experiment. The results of this experiment will be discussed in the results section of this paper.

Experiment 2

Spodoptera exigua, more commonly known as the beet armyworm, is an extremely difficult pest to control. Very few eco-friendly pest control methods are effective at warding off these pests, and as a result, individuals resort to the use of harmful chemical pesticides to solve this problem. However, entomopathogenic fungi may be an effective solution. Both *M. anisopliae* and *Paecilomyces fumosoroseus* are well-researched entomopathogenic fungi and are both eco-friendly contenders for pesticide replacements. As a result, researchers decided to test their effectiveness against the beet armyworm (Han *et al*., 2018). They obtained the pests and kept them at a temperature of 25±1°C. Then, they isolated the two fungal strains and cultivated Chinese cabbages grown in a greenhouse for 30 days. Afterwards, they transferred 600 mcL each of both strains and sprayed them on each side of the leaf. The results of this experiment will also be discussed in the results section of this paper.

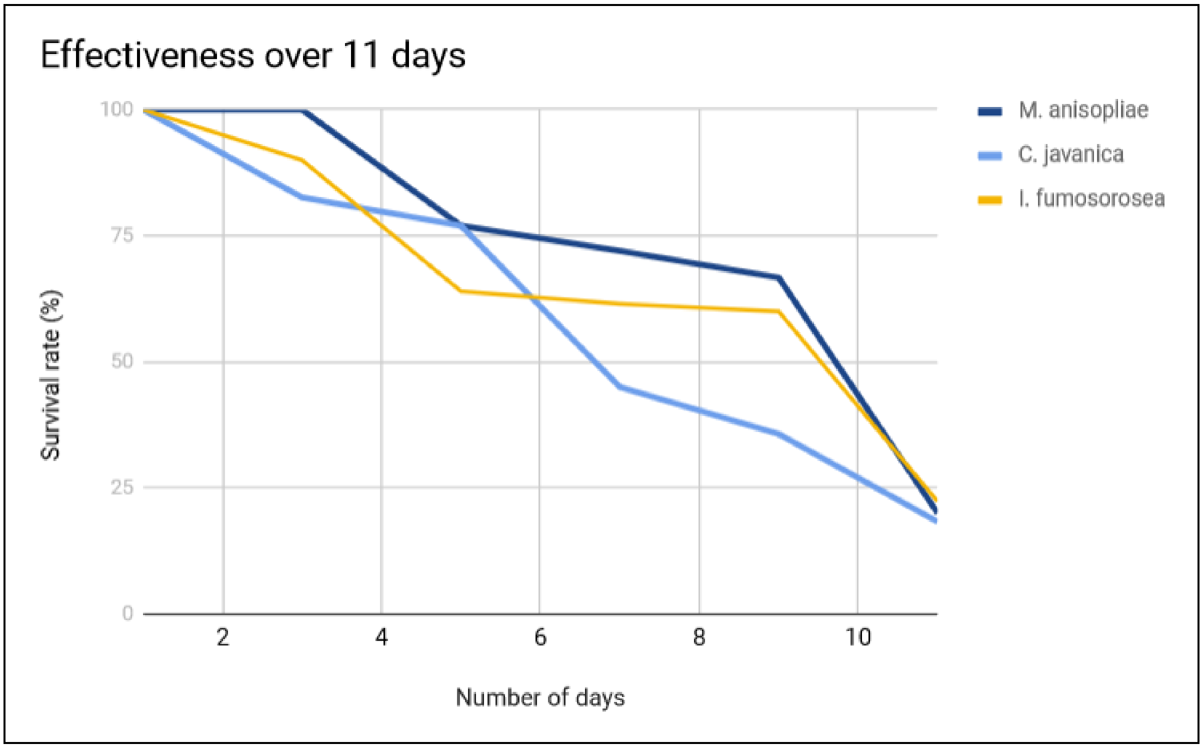
Experiment 3

Cordyceps militaris, typically known for its medicinal benefits, has been considered as a potentially effective form of eco-friendly pest control since many species within its order are entomopathogenic. To test this , researchers cultivated the spores of *C. militaris* and *B. bassiana*, and obtained Colorado potato beetle larvae (Kryukov *et al*., 2013). They grew the larvae in a 25°C, 300mL container and infected one container with *C. militaris* and the other with *B. bassiana*. After observing the mortality rate, they documented their findings for 10 days. In each experiment, at least 30 insects were used, as there was a control group, a *C. militaris* group, and a *B. bassiana* group. The results of this experiment will also be discussed in the results section of this paper.

RESULTS

Experiment 1

The survival rates measured in the Asian citrus psyllid demonstrated the effectiveness of *M. anisopliae*, *C. fumosorosea*, and *C. javanica* after 1, 3, 5, 7, 9, and 11 days, respectively (*fig. 1*). After the first three days, *C. javanica* exhibited the lowest survival rate, followed by *C. fumosorosea* and *M. anisopliae*, respectively. Throughout the first nine days, *M. anisopliae*

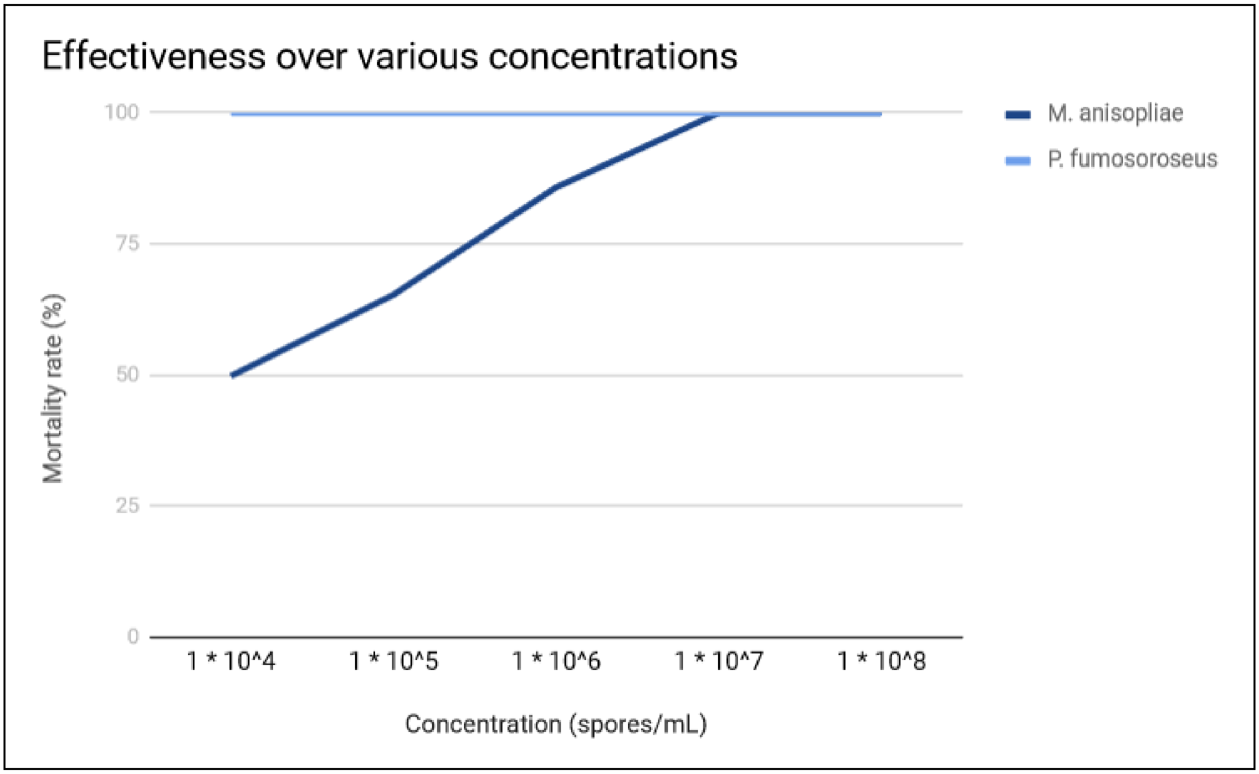


consistently lagged behind the other two strains of entomopathogenic fungi, only to observe a 46.7% drop in survival rate of the Asian citrus psyllid on the eleventh day. *C. fumosorosea* and *C. javanica* were somewhat close in terms of survival rate for the first five days, but *C. javanica* proved to be extremely effective throughout the remaining days.

Experiment 2

The mortality rates of *S. exigua* are extremely high when treated with both *M. anisopliae* and *P. fumosoroseus*.

When treated with $1x10^7$ and $1x10^8$ spores/mL, both species exhibited a 100% mortality rate against the beet armyworm. However, for lower concentrations (i.e. $1x10^4$, $1x10^5$, and $10x10^6$

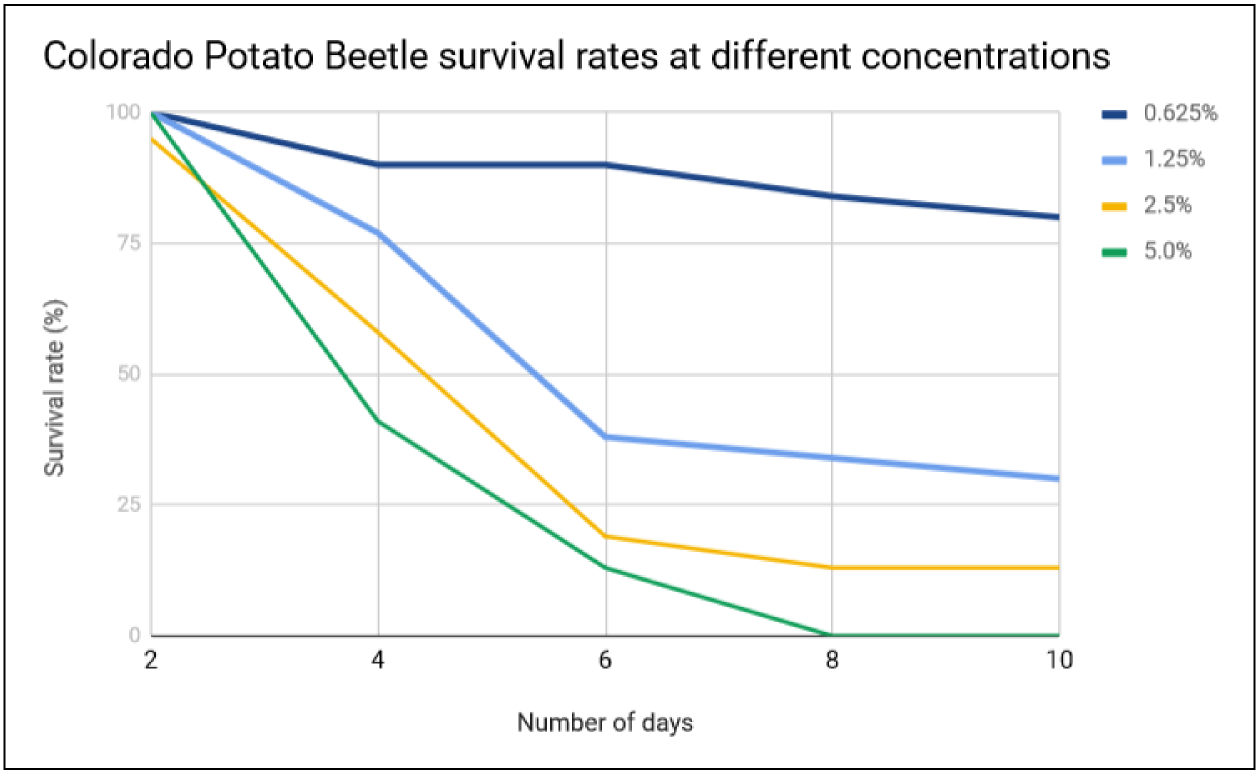


spores/mL), *P. fumosoroseus*' mortality rate was 50.2%, 34.7%, and 14.2% higher than that of *M. anisopliae* for each respective concentration (*fig. 2)*.

Experiment 3

When treated with *C. militaris*, the survival rate of the Colorado potato beetle diminished as the dose amount increased.

At 0.625% concentration of *C. militaris*, the survival rate only decreased by 20% after 10 days. However, by using twice the amount, or 1.25%, the survival rate decreased by 50%. Furthermore, 2.5% of *C. militaris* only decreases the survival rate by 17%, but using 5.0% concentration completely eradicates the Colorado potato beetle after 8 days of use. From this



data, it can be concluded that for long-term usage, 5.0% concentration of *C. militaris* is a fast, effective method of removing pests.

DISCUSSION

From these experiments, it can be concluded that entomopathogenic fungi effectively eliminates pests from agricultural settings. All of the fungi mentioned in these experiments affected the insects. Furthermore, since the survival rate of insect hosts are affected by all the tested entomopathogenic fungi in all available concentrations, it can be concluded that those fungi are, in fact, acting as a form of pest control.

In the first experiment, all three species of fungi minimized the Asian citrus psyllid's survival rate to under 20% after 11 days. Out of *M. anisopliae*, *C. fumosorosea*, and *C. javanica*, *C. javanica* decreased the insect's survival rate the most, except on the fifth day. However, throughout the entire 11-day period, *C. javanica* was proven to be the most effective species at controlling the Asian citrus psyllid. For the first seven days, *C. fumosorosea* and *C. javanica* exhibited similar reductions in survival rate, and were consistently more effective than *M. anisopliae*. However, on the ninth day, *C. fumosorosea* and *M. anisopliae* noticed a minor change in survival rate, only to drastically lower the survival rate of the Asian citrus psyllid by over 30% on the eleventh day. From this analysis, it can be concluded that *C. javanica*, *C. fumosorosea*, and *M. anisopliae* are all effective at controlling the Asian citrus psyllid over the course of 11 days.

In the second experiment, both species of fungi diminished *S. exigua*'s survival rate across all concentrations. However, *P. fumosoroseus* was far more efficient at this specific pest's removal than *M. anisopliae*. This is because *M. anisopliae*'s concentration needed to be increased by a thousand times before matching the mortality rate of *P. fumosoroseus*. In other words, to completely eradicate all specimens of *S. exigua*, one could use *P. fumosoroseus* to eradicate a thousand groups of *S. exigua* while *M. anisopliae* could only eradicate one. This increased efficiency is what renders *P. fumosoroseus* far better against this specific pest. However, it should be noted that although *M. anisopliae* displayed a less severe effect against pests versus other species of fungi in experiments 1 and 2, it can effectively control both pests. In other words, while *M. anisopliae* may not be the most effective, it trades performance for variety, allowing it to affect a wide variety of hosts instead of just one.

In the third experiment, it can be concluded that *C. militaris* is an effective alternative to chemical pesticides. Across all concentrations, the Colorado potato beetle was affected by *C.*

militaris. As the amount of *C. militaris* increases, so does the overall efficiency. In other words, it is far more effective to use one dose of 5.0% *C. militaris* than using multiple doses of smaller concentrations that add up to 5.0%. For example, eight 0.625% doses add up to one 5.0% dose, but eight small doses is much less effective than one 5.0% dose. This is because one dose of 0.625% spore concentration removes only 20% of the population, and eight doses will result in a ~16.8% survival rate compared to 0% with one full 5.0% dose. Moreover, four 1.25% doses will result in a ~0.8% survival rate compared to a 0% rate with one 5.0% dose, making the four doses roughly equal in effectiveness. Two 2.5% doses will result in a ~1.7% survival rate. Therefore, using one 5.0% dose against the Colorado potato beetle is the most effective, followed by four 1.25% doses. Both will eradicate around the same number of pests while consuming the same amount of *C. militaris*.

Based on these experiments, it can be concluded that all species of entomopathogenic fungi are effective alternatives to pesticides. *M. anisopliae* proved to be effective in both of the experiments in which it was tested, though *P. fumosoroseus* and *C. javanica* were individually more effective. Moreover, all species of fungi tested in these three experiments belonged to the *Hypocreales* order, and most of the species were asexual or sexual forms of the *Cordyceps* genus. This indicates that the methods that *Cordyceps* species utilize to attack insects prove to be effective against a variety of pests and should be considered as pesticide alternatives.

CONCLUSION

The *Hypocreales* order is home to many species of entomopathogenic fungi, all of which deter pests effectively. These species of fungi can almost completely eradicate an insect population within ten to eleven days, and some species (such as *P. fumsoroseus*) can eliminate insects at a low concentration, proving its high performance and efficiency as a pesticide. Furthermore, these experiments shed light on biopesticide alternatives for traditional commercial pesticides which pose a threat to our health.

Growing these fungi are not relatively difficult either. With proper research and experimentation, one can obtain spores of these species in just a few weeks. Furthermore, one can also obtain these spores online and apply them to plants that are being affected by pests. Usually, these mycopesticides are in the form of spore sprays, but they can also be grown near the plants. As such, entomopathogenic fungi not only provide an effective and efficient alternative to harmful pesticides, but they are relatively easy to obtain, cultivate, and use.

Additionally, these mycopesticides do not harm the environment like chemical pesticides do. These mushrooms have evolved to live with non-host organisms and are therefore safe for humans and other organisms besides insects living in the ecosystem where the fungi is applied. Furthermore, the method mycopesticides use to attack insects ensures that one application of the insecticide is sufficient for a long period of time since the fungal spores are expelled to cover a large area after a victim is decimated. In other words, as the fungi kill insects, its spores are continuously distributed to kill even more insects.

Overall, entomopathogenic fungi presents a promising, effective and efficient solution to chemical pesticides. It impacts a wide variety of insect hosts, offers a powerful, complex method to attack and eliminate an insect population, and has a harmonious relationship with the wildlife around it. Therefore, these mycopesticides should be considered as a replacement for commercial pesticides for gardening and agriculture.

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