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PHYTOPATHOGENIC ORGANISMS AND MYCOTOXIGENIC FUNGI: WHY DO WE CONTROL ONE AND NEGLECT THE OTHER? A BIOLOGICAL CONTROL PERSPECTIVE IN MALAYSIA

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ABSTRACT

In this review, we present the current information on development and applications of biological control against phytopathogenic organisms as well as mycotoxigenic fungi in Malaysia as part of the integrated pest management (IPM) programs in a collective effort to achieve food security. While the biological control of phytopathogenic organisms of economically important crops is well established and widely practiced in Malaysia with considerable success, the same cannot be said for mycotoxigenic fungi. This is surprising since the year round hot and humid Malaysian tropical climate is very conducive for the colonization of mycotoxigenic fungi and the potential contamination with mycotoxins. This suggests that less focus has been made on the control of mycotoxigenic species in the genera *Aspergillus*, *Fusarium* and *Penicillium* in Malaysia, despite the food security and health implications of exposure to the mycotoxins produced by these species. At present, there is limited research in Malaysia related to biological control of the key mycotoxins, especially aflatoxins, *Fusarium*-related mycotoxins and ochratoxin A, in key food and feed chains. The expected threats of climate change, its impacts on both plant physiology and the proliferation of mycotoxigenic fungi, and the contamination of food and feed commodities with mycotoxins, including the discovery of masked mycotoxins, will pose significant new global challenges that will impact on mycotoxin management strategies in food and feed crops worldwide. Future research, especially in Malaysia, should urgently focus on these challenges to develop IPM strategies which include biological control for minimizing mycotoxins in economically important food and feed chains for the benefit of ensuring food safety and food security under climate change scenarios.

Keywords: mycotoxin; *Aspergillus*; *Fusarium*; *Penicillium*; biocontrol, climate change

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1. Biological control and food security

Feeding the global population of nearly eight billion people is indeed the most daunting challenge facing mankind in the modern world. Food security, which is defined as "…when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences to support an active and healthy life…", is the primary concern in addressing this challenge (FAO, 2009). Since time immemorial, agriculture and aquaculture have been, and still are, the primary nutritional support to mankind, and acts as the first pillar of food security, which is "food availability" (*i.e.*, supply of food through production, distribution, and exchange). The other three pillars according to Gregory et al. (2005) are "food accessibility" (*i.e.*, affordability and allocation of food, as well as the preferences of individuals and households), "food utilization" (*i.e.*, consumption of safe, quality and sufficient food for human health and metabolism) and "food stability" (*i.e.*, ability to obtain food over time). However, the continual growth in the human population and competition for land, water and energy, will eventually and inevitably impair our agricultural ability and capacity to produce sufficient food to feed this increasing global demand (Godfray et al., 2010). What is more, the global agricultural sector is also constantly left at the mercy of its old arch enemy– pests and pathogens! It was recently estimated that 40-50% of crop yields in developing countries are wastefully lost to pests and crop diseases pre- and post-harvest. For developed countries such as the United States, the estimate stands at 20-25% (NIFA, 2015). Apart from pests and crop diseases, weeds can also thrive in monoculture systems, thus significantly impacting on the agricultural yields (Poggio, 2005).

Pest is a broad term frequently used and defined as any species or strain of living organism (*e.g.*, animals, plants, microorganisms), usually occurring in high density in a specific niche, which results in detrimental and injurious effects on humans, animals or plants. In agriculture, pests are regarded as the key biotic component which can adversely impact on agricultural yields and productivity along the supply chain, with the major abiotic component being the climate. To mitigate this, the management or eradication of crop pests of economic importance has been practiced worldwide by using various control measures. There are even documents citing that pest control is as old as agriculture itself since it has always been necessary to keep the crops pest- and disease-free (Oerke, 2006). The various control measures of pests are generally divided into three– physical / mechanical, chemical and biological, and have been employed either individually or collectively before, during or after crop cultivation as part of an Integrated Pest Management (IPM) strategy and Good Agricultural Practice (GAP).

Physical or mechanical pest control involves the use of simple equipment and devices as well as hands-on techniques that form a protective barrier between the crops and the pests (Vincent et al., 2003; Khan & Ram, 2014; Sorensen et al., 2016). These include controlled irrigation to minimize stresses on the crops, crop rotation, crop monitoring, growing pest-resistant crop varieties / cultivars through selective breeding, intercropping, seed selection, soil ploughing, the use of physical barriers, nettings or trappings, timely harvesting, and trap cropping (*i.e.*, companion crops planted nearby the main crops (companion planting) to divert pests away from infesting the main crops; Holden et al., 2012).

Chemical pest control, on the other hand, involves the use of chemically-synthesized pesticides which can be employed either as solid (*e.g.*, by luring the pests to eat chemically-poisoned baits), liquid (*e.g.*, by spraying formulated pesticide suspensions onto the crops or soil), or gaseous (*e.g.*, by suffocating pests with chemically-poisoned fumigants). Apart from chemicals, there are also plant-derived / plant-based control agents known as phytochemicals which pose lesser threat to the environments and human health as compared to their more recalcitrant chemically-synthesized counterparts. As critically reviewed by Koul (2008), these phytochemicals, which include alkaloids, chromenes, cucurbitacins, cyclopropanoid acids, phenolics, polyacetylenes, quassinoids, saponins, terpenes, and their various derivatives, are usually applied as antifeedants and/or deterrents. Additionally, there are also semiochemicals (*e.g.*, pheromones, allelochemicals) which are defined as substances used in plant-insect or insect-insect interactions as complementary components to insecticide approaches in IPM. Semiochemicals are used to effectively modify insect pests' behavior by affecting their survival and/or reproduction in the effort to control their infestations on crops (El-Ghany, 2019).

Another alternative is biological control, or simply biocontrol, which is a technique to significantly reduce or permanently suppress the population density of pests to a level which has no significant effect on the crop yield by interfering with the pests' ecophysiology using living organisms (Wyckhuys et al., 2013). If properly applied, biological control could be a long-term and self-sustaining treatment method for managing pests (Sahayaraj, 2014) which will consequently contribute to lower agro-operational costs. At present, there are four widely accepted fundamental strategies in biological control (van Lenteren et al., 2018): (1) *natural*, in which natural enemies of the pests are not frequently noticed and consciously manipulated by humans but their presence actually helps to regulate the population density of their host or prey; (2) *importation* (classical), in which foreign / exotic natural enemies of the pests are intentionally reared and released into new habitats or ecosystems to regulate the population density of their host or prey; (3) *augmentation*, in which natural enemies of the pests, that are unable to survive and/or persist in the new habitats or ecosystems, are reared in large population and mass-released (inundative augmentation) or periodically released in smaller numbers (inoculative augmentation) to regulate the population density of their host or prey; and (4) *conservation*, in which natural enemies of the pests are aided (*e.g.*, providing them with shelters, reducing the use of chemicals that might kill them) to maintain and optimize their survival and/or effectiveness in regulating the population density of their host or prey. Figure 1 summarizes the principles and approaches of Integrated Plant Protection (IPP) otherwise also frequently referred to as IPM (Frische et al., 2018). The IPP or IPM pyramid in Figure 1 ideally works from the bottom up, in which preventive measures (*e.g.*, the use of tolerant / resistant crop cultivars; crop care such as farm irrigation and crop rotation; promoting natural antagonists against crop pests) always take priority and precedent before the use of chemicals. Only when the economic threshold is exceeded, as assessed by the risk analysis and monitoring, would the plant protection approach shift to biotechnological measures (*e.g.*, biological, physical, chemical controls). An "economic threshold" is the pest's population level infesting the farm or the extent of crop damage inflicted by the pests at which the value of the crop destroyed exceeds the cost of controlling the pest (Zalom, 2010).

Of course as climate is in a state of flux and the pests rapidly evolve, these control measures will often have flaws and drawbacks and have to be appropriately modified. For example, the physical control on a much larger scale requires enormous time and manpower which will most likely contribute to higher operational costs, while the chemical control has long been associated with high production and application costs, human health hazards, restriction by domestic and international regulatory limits, trade bans, residual effects, environmental pollution, resistance development in pests and potential elimination of beneficial natural enemies of the targeted pests (Bommarco et al., 2011). Also, as illustrated in Figure 1, a good and sustainable IPM strategy always uses chemicals as the last resort. As for biological control, several drawbacks have also been highlighted such as they do not function as rapidly as chemical pesticides, their application requires special skills / knowledge, or even worse, they prey on beneficial organism thus disrupting the natural food chain. To mitigate the latter, care and consideration should be taken into account when developing an efficient and successful biological control system. Simberloff in 2012 outlined four potential risks when introducing a biological control agent: (1) direct attack on non-targets; (2) indirect effects on non-targets (*e.g.*, competition); (3) dispersal of the introduced biological control agent to other area; (4) changed relationships between the introduced biological control agent and the native species (*e.g.*, when the introduced biological control agent multiply uncontrollably and becomes invasive). Of course the risk of non-target is always there (also known as the “non-target effect”; Louda et al., 2003) and has been heavily debated; which is why risk analysis and assessment (*e.g.*, host specificity test; Kaufman & Wright, 2017) must be critically performed before a biological control agent is introduced in the crop agro-ecosystem. Köhl et al. (2011) proposed nine sequential steps in selecting, developing and introducing a biological control agent of plant pathogens: (1) assessment of targeted crop, disease and markets, (2) isolation of candidate antagonists, (3) rapid-throughput screening in terms of production, safety, and ecology, (4) database mining in terms of intellectual property (IP) protection, safety, ecology, environmental risks, and marketing, (5) efficacy testing in bioassays, (6) preliminary assessment of mass production, (7) pilot formulation, (8) upscaling mass production and full field testing, and (9) integration into cropping systems with constant monitoring of its efficacy and environmental risks. These are critically performed to prevent the disadvantages of introducing a biological control agent outweighing its advantages. Furthermore, as previously mentioned, biological control is never applied individually but collectively with other measures as part of IPM or GAP programs.

In Malaysia, agriculture is the third engine that drives and sustains the economic growth after services and manufacturing in terms of gross domestic product (GDP), with oil palm (*Elaeis guineensis*) and rice (*Oryza sativa*) dominating the production scene (Fahmi *et al*., 2013). Although the industrial commodity sub-sector (*e.g.*, cocoa, palm oil, rubber, timber) serves as the primary contributor to the sector’s GDP, the growth rate of the food commodity sub-sector (*e.g.*, fruits, rice, vegetables) is gradually increasing which indicates a positive improvement of the domestic food production while reducing import dependency (Ahmad & Suntharaligam, 2009). Moreover, the approach recently undertaken by the government to further increase the domestic food production to feed the 32 million population has shifted from the expansion of land for animal rearing and crop cultivation to sustainable agricultural intensification in which farmers are encouraged to use fewer inputs (*e.g.*, energy, water, nutrients, pesticides) and achieve higher productivity within similar acreage of land. Figure 2 illustrates the

agricultural production in Malaysia in 2017. Like many other countries, Malaysian agricultural productivity is also impacted by pest infestation, against which the country has launched numerous measures in a collective effort to combat further deterioration. Among such measures is biological control.

Biological control has a long history in Malaysia. The earliest available online resource dates back to 40 years ago in 1978 on the use of the beetle *Metrogaleruca obscura* and the wasp *Eurytoma attiva* to control the invasive weed *Cordia curassavica* (tropical black sage) through defoliation and seed destruction, respectively (Simmonds, 1980). Since then, Malaysian agricultural authorities, particularly the Department of Agriculture (DOA) and Malaysian Agricultural Research and Development Institute (MARDI), both under the jurisdiction of the Ministry of Agriculture and Agro-based Industry, has been conducting numerous research initiatives to combat pest infestation on economically important crops such as oil palm and rice paddy. Although it is understandable why the focus has always been, and still is, on the control of phytopathogenic organisms, it must not be forgotten that pests come in different forms. Since the discovery of T-2 toxin that caused alimentary toxic aleukia in Russia in the 1940s (Williams, 1989; Joffe, 1971), and aflatoxins that killed nearly 100,000 turkey poults in England in the 1960s (Blount, 1961; Wannop, 1961), the global scientific community has been alarmed by the disease- and death-causing capability of fungal secondary metabolites which can affect both humans and animals (Pitt & Miller, 2017). These secondary metabolites were henceforth termed mycotoxins (from the Greek *mykes* which is fungus, and *toxikon* which is poison), and their fungal producers are known as mycotoxigenic fungi. Being produced by several genera of microfungi, mycotoxin contamination is in fact the secondary outcome of fungal infestation of crop commodities, with the primary ones being the damages and diseases such as blights, rusts, rots and wilts. Although just a secondary outcome, the clinical effects of mycotoxins on humans and animals are certain with several types of mycotoxins being classified as carcinogens (IARC, 2019; Ostry et al., 2017). The ecophysiological conditions influencing mycotoxigenic fungal growth and mycotoxin production as well as major mycotoxins and their associated health risks on humans and animals have been extensively reviewed elsewhere (Bhat et al., 2010). Nevertheless, despite Malaysian climatic conditions (*i.e.*, tropical; warm and humid all year round) being highly favorable for fungal proliferation, including that of mycotoxigenic fungi, on staple crop commodities pre- and post-harvest, to the best of our knowledge thus far, there has been very little, if any, research related to the use of biological control agents to control mycotoxigenic fungi and their associated mycotoxin contamination in Malaysia. The present review therefore discusses (i) the application of biological control agents in regulating the infestation of phytopathogenic organisms in Malaysia, (ii) the current status of biological control against mycotoxigenic fungi in Malaysia and the potential reasons for their lack of use, and (iii) the future directions of biological control research in Malaysia, with a focus on mycotoxigenic fungi and mycotoxin control.

2. Biological control against phytopathogenic organisms

Although regarded as a newly-industrialized country (*i.e.*, transitional phase between developing and developed countries), Malaysia still relies on the agriculture sector which is the nation's third economic engine. The First (1984-1991), Second (1992-1997) and Third National Agricultural (1998-2010) Policies helped to shape the sector to be productive, sustainable and competitive in the face of agricultural challenges in a rapidly changing world. Under the various long-term and far-sighted initiatives

undertaken by the Ministry of Agriculture and Agro-based Industry, crops are being cultivated on a larger scale and are usually well managed. Approaches such as IPM and GAP – which among others include regular monitoring, timely harvesting, supplementation and utilization of natural control agents and the prudent use of chemicals – are being put into practice in order to improve yield, safety and quality of the agricultural products.

As part of a good IPM, biological control of phytopathogenic organisms has been widely applied in Malaysia with all the four main biological control strategies (*i.e.*, *natural*, *importation*, *augmentation*, *conservation*) have been documented. The role of natural enemies in controlling native pests has been recognized in economically important crops especially oil palm, cocoa, coconut and rice. The introduction of parasitoid wasps *Diadegma semiclausum* and *Diadromus collaris* in the 1970s to control *Plutella xylostella* (diamondback moth whose larvae destructively infest cruciferous vegetables especially cabbages) had suppressed the drawbacks of the reduction in chemical pesticide usage (Ooi, 1992; Ooi & Lim, 1989). The introduction of beneficial plants such as *Cassia cobanensis* (cassia), *Turnera subulata* (white buttercup) and *Antigonon leptopus* (Mexican creeper) has provided shelter and supplementary food such as nectar, and encouraged the proliferation of predators and parasites (Jamian, 2017). The conservation of natural or introduced enemies has also been important in insect pest management in Malaysia.

Table 1 summarizes almost 40 years of research on the biological control agents, their targeted pests / pathogens / diseases, and their potential mechanisms of action against phytopathogenic organisms in Malaysia. Figure 3 further illustrates the distribution of the targeted crop commodities on which research on potential biological control agents against phytopathogenic organisms have been focused. From Figure 3, it is apparent that oil palm – being the country's foremost crop in the agriculture sector – evidently receives appreciable attention and emphasis in terms of protection and prevention against diseases. The major pests of oil palm in Malaysia include bagworm moths (*Metisa plana*, *Pteroma pendula*), bunch moths (*Tirathaba rufivena*), rats, rhinoceros beetles (*Oryctes rhinoceros*), and termites (*Coptotermes curvignathus*), while the most important diseases are *Ganoderma* basal stem rot (caused by *Ganoderma boninense*) and *Marasmius* bunch rot (caused by *Marasmius palmivorus*) (SALCRA, 2019). To mitigate the *Ganoderma* basal stem rot, the Malaysian Palm Oil Board (MPOB), a government agency responsible for the research and development of palm oil industry in Malaysia, has introduced the Integrated *Ganoderma* Management (IGM) which incorporates several elements including sanitation, biological control, chemical control and the use of fertilizers with beneficial trace elements. The integration of biological control in the system is based on the understanding that low incidence of disease has been achieved in the presence of natural competitors / predators that keep the parasitic fungi under control (Bivi et al., 2010). In an effort to further strengthen this approach, MPOB has also developed and commercialized their biological control formulations namely GanoEF bio-fertilizer (contains endophytic *Hendersonia* GanoEF1 fungus, mechanism of action: production of inhibitory bioactive compounds, Nurrashyeda et al., 2015); and Embio^{TM} actinoPLUS (contains *Streptomyces* sp., mechanism of action: production of inhibitory bioactive compounds, Shariffah-Muzaimah et al., 2015).

For rice paddy, the rice blast disease caused by *Pyricularia oryzae* is considered a major endemic in rice paddy planting areas in Malaysia and across the world.

Currently, the cultivation of resistant varieties and the application of fungicides are the most commonly used methods in controlling the disease in rice paddy planting areas in Malaysia (Abed-Ashtiani et al., 2018). However, due to the fact that both practices fail to provide a long term solutions for the disease control, growing amounts of research have been conducted to incorporate biological control approach as an alternative. Recently, an endophytic actinomycete, *Streptomyces* sp. strain UPMRS4, isolated from the rhizosphere of irrigated rice paddy fields in Malaysia, has been shown to successfully suppress the rice blast disease through the production of inhibitory bioactive compounds, thus inducing the systemic resistance of rice paddy against *P. oryzae* (Awla et al., 2017).

Figure 4 depicts the types of potential biological control agents against phytopathogenic organisms in Malaysia, which are dominated by bacteria, ascomycetes and insects. The use of bacteria as biological control agents, especially *Bacillus* spp., *Burkholderia* spp. and *Pseudomonas* spp., has shown considerable success in Malaysia (Table 1). Ubiquitous in nature, these bacteria exist as both non-parasitic and parasitic pathogenic species; the latter as potent producers of various enzymes and antimicrobial substances that inhibit important phytopathogens while at the same time promote plant growth by the production of volatile substances (Beneduzi et al., 2012).

Furthermore, considering that the majority of crop pests are insects, the use of entomopathogenic fungi, especially from the Division Ascomycota, has proven efficient on many crop species (Strasser et al., 2010). Species like *Beauveria bassiana*, *Isaria fumosorosea*, *Metarhizium anisopliae*, *Paecilomyces* spp. and *Trichoderma* spp. have been efficacious against beetles, moths and termites infesting a multitude of crop commodities in Malaysia (Table 1). The tropical climate of Malaysia enhances the propagation and proliferation of these entomopathogenic fungi. In lethally parasitizing the target insect pests, or severely disabling them, entomopathogenic ascomycetes usually initiate the infection in the form of airborne conidia which naturally or artificially (*i.e.*, through human intervention) land onto the insects' exoskeleton. Under suitable ecophysiological conditions, these spores germinate and grow into hyphae, thereby colonizing the insect's cuticle. These hyphae are capable of producing hydrolyzing enzymes which bore a tunnel through which the hyphae could penetrate into the insects' body cavity, and there proliferate further, thus killing the host (Fernandes et al., 2012). Entomopathogenic fungi can grow over a wide range of temperatures, and some strains have the appropriate ecophysiological growth parameters for use in climates such a Malaysia (Borisade & Magan, 2015). Being environmentally safe, the use of entomopathogenic ascomycetes draws worldwide interest for biological control of insect pests including in Malaysia. Apart from insects, these entomopathogenic fungi have also been shown to parasitize other phytopathogenic ascomycetes as well as nematodes (Table 1).

For insect species that are used to control insect pests, the biological control agents come either as insectivorous predators or insect parasitoids. Insectivorous predators are mainly free-living species that aggressively prey on a large number of insects during their lifetime, thus controlling the pest density (Eggleton & Belshaw, 1992). Given that many major crop pests are insects, majority of the biological control agents used are insectivorous species (Table 1). The ladybird beetles (*Menochilus sexmaculatus*) are voracious predators of the aphids (*Rhopalosiphum maidis*) infesting

maize. Various predatory ant species such as *Dolichoderus thoracicus* have been successful in controlling the cocoa pod borer moths (*Conopomorpha cramerella*) whose larvae infest cocoa, and mosquito bugs (*Helopeltis theobromae*) which cause leaf tattering and fruit blemishes in cocoa; and *Oecophylla smaragdina* which prey on bagworm moths (*Pteroma pendula*) whose larvae infest oil palms, and *Hypsipyla robusta* whose larvae feed on timber shoots, flowers and barks.

For insect parasitoids, their mechanism of action is generally by laying their eggs (oviposition) on (or in) the body of an insect host, on (or in) which their developing larvae will feed, consequently decapitating the host (Kapranas et al., 2012). Parasitoids are almost always endopterygote insects which have complete metamorphosis stages during their lifecycle; as parasitoid larvae and free-living adults. Parasitoids are generally classified into endoparasitoids which live within their host's body, or ectoparasitoids which feed on their host's body from outside (Colmenarez et al., 2018). The majority of insect parasitoids that have been used in Malaysia are from the wasp family such as *Acerophagus papayae*, *Apanteles metesae*, *Cotesia vestalis*, *Diachasmimorpha longicaudata*, *Fopius arisanus*, *F. vandenboschi*, *Psyttalia incisi*, *P. fletcheri* and *Tamarixia radiata* which have shown considerable success in reducing the density of moth caterpillars, bugs, fruit flies and psyllids infesting various economically important fruits and vegetables such as cabbages, carambolas (star fruit), melons, oranges and papayas (Table 1). Wasps have been shown to exhibit both endo– and ectoparasitoid behaviors.

Although the majority of insect parasitoids are wasps (Hymenoptera) and flies (Diptera) (Eggleton & Belshaw, 1992), to the best of our knowledge thus far, there has been no available record on the use of the latter as a biological control agent in Malaysia. This could either be due to the fact that this particular application is totally new in Malaysia, therefore suitable for future investigation; or that ongoing trials are not yet conclusive and published.

3. Biological control against mycotoxigenic fungi

Mycotoxins are low molecular weight and heat stable secondary metabolites produced by microfungi, and capable of causing diseases and death in vertebrates (humans and animals; Bennett & Klich, 2003). Members of the genera *Aspergillus*, *Fusarium* and *Penicillium* are often regarded as the major producers of mycotoxins with health and economic impacts. Among the major challenges in controlling mycotoxigenic fungi and the subsequent mycotoxin contamination in crop commodities is that they are not very sensitive / susceptible to fungicides commonly used in the field to control fungal pathogens. At present, almost 500 different mycotoxins and fungal metabolites have been identified as being produced by more than 300 microfungal species (Nielsen & Smedsgaard, 2003). Although mycotoxins are not necessary for growth of their fungal producers, but because mycotoxins can weaken their competitors (usually other microorganisms competing for the same substrate on crop commodities; competitive exclusion), the fungal producers are thought to use mycotoxins as a strategy to ensure their survival and proliferation (Hussein & Brasel, 2001). In terms of their carcinogenicity towards humans and animals, the International Agency for Research on Cancer (IARC), which is under the auspices of the World Health Organization (WHO) of the United Nations (UN) and serves as the authoritative source of information on causes of cancers (IARC, 2019), has classified mycotoxins into several groups (Ostry et al., 2017) as in Table 2.

Although the biological control against phytopathogenic organisms has a long history in Malaysia, as mentioned earlier, the same is not entirely true for mycotoxigenic fungi. Surprisingly, the application of biological control against mycotoxigenic fungi is almost non-existent in Malaysia with only a handful of publications available online on the subject matter (Samsudin & Magan, 2016; Samsudin et al., 2016; Samsudin et al., 2017). These studies examined the use of *Clonostachys rosea* and *Streptomyces* sp. to control the growth of *Fusarium verticillioides* and the contamination of maize with fumonisins. The only other study of similar nature was published slightly earlier on the use of the mycoparasite *Trichoderma harzianum* against *F. proliferatum* and *F. verticillioides* in controlling *Fusarium* ear rot in maize (Suhaida & NurAinIzzati, 2013). However, they did not specifically examine or quantify the impact on control of any mycotoxins. Since these fungal pathogens, under Malaysian climatic conditions, would certainly contaminate maize with fumonisins during silking, this would have been useful additional information. It is possible that this has not been recognized as an important research area in Malaysia so far. However, as Malaysia does have legislations for mycotoxin contamination of some commodities (Table 3; Malaysian Food Regulation, 1985), this could be a major driver for practically related research for the reduction of mycotoxins using biological control strategies in the very near future. Table 4 lists the occurrence data of mycotoxins on Malaysian foods and feedstuffs, from which it is apparent that the concern is more on aflatoxins. Malaysia's tropical climate, which is favorable for *Aspergillus* spp. proliferation and the subsequent mycotoxin production, poses a constant threat to its agriculture sector in producing safe and quality harvest. A more comprehensive review on mycotoxin contamination of Malaysian food and agricultural produce has been published elsewhere (Afsah-Hejri et al., 2013). The fact that peanuts and maize are the two most contaminated crop commodities by members of *Aspergillus* section *Flavi* (Samson et al., 2010; Pitt & Hocking, 2009) which almost invariably produce the Group 1 carcinogenic aflatoxins, and that these two are relevant in Malaysian agricultural sector (listed as cash crops; Figure 2), further investigation on the development of an efficient control against aflatoxigenic fungal colonization on these crops is therefore highly warranted. Similar threat on peanuts and maize is also observed in the Philippines (Balendres et al., 2019), Ghana (Agbetiameh et al., 2018), and Zambia (Kachapulula et al., 2017).

As described in Table 1, several biological control agents have been investigated on their effects against phytopathogenic *Fusarium* spp. More detailed examination shows that some of these *Fusarium* spp. are in fact also mycotoxigenic (*e.g.*, *F. proliferatum:* beauvericins, fumonisins, moniliformin; *F. solani:* moniliformin, trichothecenes; *F. verticillioides:* fumonisins, fusaric acid, moniliformin) according to Desjardins (2006). Therefore, opportunities do exist for replicating such studies with biological control agents and investigate their effects in controlling mycotoxin contamination. In addition, as most studies have focused on the use of effective biological control agents against plant diseases, it may be beneficial to also screen these for mycotoxin control (Medina et al, 2017a). Nevertheless, previous studies have also suggested that several potential bacterial antagonists may, under certain abiotic conditions, stimulate mycotoxin production instead of inhibiting it (Al-Saad et al., 2016). Thus, care is needed when screening for mycotoxin control. There are also some cases in which the fungal pathogens could "disarm" the applied antagonist. For example, it was shown that deoxynivalenol-producing *F. culmorum* and *F. graminearum* strains could repress the expression of genes that regulate the chitinase enzyme production in the biological

control agent *Trichoderma atroviride* (Lutz et al., 2003). This is not unusual since pathogens also possess diverse responses to counteract antagonists, which include detoxification and active efflux of antibiotics, and repression of biosynthetic genes involved in biological control (Duffy et al., 2003).

In other parts of the world however, the application of biological control agents to combat mycotoxigenic fungi is more widely practiced. Among the earliest and excellent reviews that discussed the potential use of non-toxigenic strains of fungi, yeasts and bacteria in controlling mycotoxigenic fungi was published by Cleveland et al. (2003). The review also discussed the indirect controls of mycotoxigenic fungi by controlling their vectoring insects or developing insect-resistance plant cultivars (*e.g.*, Bt maize) since insect damage provides entry points for mycotoxigenic fungi to colonize the crop, thus resulting in increased mycotoxin contamination. Recently, a review on the dynamics and mechanisms of action of biological control agents against mycotoxigenic fungi was critically discussed by Medina et al. (2017a). They suggested that the mechanism of action and the inoculum dose required for effective control of mycotoxigenic fungi may be different from that for phytopathogenic fungi. Therefore, economical production / formulation is critical for successful biological control of mycotoxins. Nevertheless, they concur that the primary mechanisms of action for the control of mycotoxigenic fungi or phytopathogenic organisms usually include niche / habitat exclusion, competition for nutrients and production of inhibitory volatiles. In fact, Köhl et al. (2019) have suggested that biological control agents utilize a cascade of different interacting approaches for efficacy. Based on the available literature, we could summarize the biological control agents into three major groups; (1) non-toxigenic strains of known mycotoxin producers, (2) mycoparasitic microfungi, and (3) phyllosphere or soilborne bacteria.

The use of non-toxigenic *Aspergillus flavus* and *A. parasiticus* strains to control their toxigenic counterparts began in the early 1990s in the USA (Brown et al., 1991). Since then, this application has found success in other parts of the world where aflatoxin contamination is significant and has had significant impacts on the economies, such as Kenya (Marchera & Ndwiga, 2015), Italy (Mauro et al., 2018), Argentina (Alaniz Zanon et al., 2018) and Thailand (Tran-Dinh et al., 2018). Its modes of action is either through competitive exclusion (*i.e.*, toxigenic strain displacement) (Damann 2015), or through thigmoregulation (*i.e.*, touch inhibition) (Huang et al., 2011). However, in securing an efficient biological control agent, it should be noted that lack of mycotoxin biosynthesis because of the deletion of key biosynthetic gene clusters may not be the only criterion; rather the non-toxigenic candidate must also be an effective colonizer of the crop commodity onto which it would be applied (to competitively exclude its competitors), and that it must also be able to reduce the mycotoxin contamination (Horn & Dorner, 2011). Recent investigation proposed yet another mechanism for the antagonism of the non-toxigenic strains which is via active extrolites (*i.e.*, uncharacterized compounds) secretion (Moore et al., 2019).

Another successful area for research and applications is the use of mycoparasitic microfungi. As described in Table 1, *Trichoderma* spp. feature predominantly as a competent biological control agent against a wide variety of phytopathogenic organisms. Similar traits are also exhibited against mycotoxigenic fungi and subsequent mycotoxin production (Veenstra et al., 2019; Braun et al., 2018; Saravanakumar et al., 2018). Prevalently found in the rhizo- and phyllosphere, the free-

living and fast-growing *Trichoderma* spp. often exist in mutualistic endophytic relationships with plants. As a biological control agent, *Trichoderma* spp. demonstrate antagonism through antibiotic production, parasitism (*e.g.*, penetration, killing), inducing host-plant resistance (*i.e.*, *Trichoderma* spp. are also known to have plant growth promoting characteristics), and habitat / nutrient competition (*i.e.*, *Trichoderma* spp. are effective decomposers and aggressive colonizers, thus leaving scarce macro- and micro-nutrients from the soil for other competitors). *Trichoderma* spp. also produce a wide array of enzymes, chief among which is the fungal-cell-wall-degrading chitinase (Harman, 2006). The ability of *Trichoderma* spp. to produce chemically diverse secondary metabolites that could modify the environmental conditions into that which is inhospitable for their competitors has also been extensively reviewed by Keswani et al. (2014) and Reino et al. (2008).

Finally is the use of soil-borne bacteria that produce various antibiotics and fungal-cell-wall-degrading enzymes. As in the control of phytopathogenic organisms, *Bacillus* spp. also exhibit potency and prowess in the control of mycotoxigenic fungi. This is mainly attributed to the production of cyclic lipopeptides which display amphiphilic properties that could disrupt the fungal cell wall and cell membrane (Siahmoshteh et al., 2017). Other chemical metabolites produced by *Bacillus* spp. could also inhibit fungal nutrient acquisition, mycelial growth and conidial germination (Volpon et al., 2000: Li et al., 2009; Cao et al., 2012). Recent investigation also elucidates the ability of enzymes produced by *Bacillus* spp. in degrading mycotoxins (Afsharmanesh et al., 2018). Besides *Bacillus* spp., lactic acid bacteria have also been shown to possess antimycotoxigenic properties, including the production of organic acids which will acidify environmental pH, thus inhibiting fungal growth and mycotoxin production; antifungal compounds (*e.g.*, propionate, cyclic dipeptide); and competitive exclusion of fungal pathogens (Guimarães et al., 2018; Oliveira et al., 2014). The efficiency of *Streptomyces* spp. in combatting mycotoxigenic fungi is also of note. The production of various bioactive molecules such as antibiotics (*e.g.*, candicidin, phosphlactomycin, tubercidin, pyrrole-2-carboxylic acid), lytic enzymes and volatile organic compounds (*e.g.*, phenylethyl alcohol, (+)-*epi*-bicyclesesquiphellandrene) has greatly contributed to the antagonism (Mari et al., 2016; Nguyen et al., 2018; Hoster et al., 2005). The growth inhibition of mycotoxigenic fungi and the subsequent mycotoxin production by *Streptomyces* spp. in wheat (Palazzini et al., 2017; Palazzini et al., 2007; Nourozian et al., 2006) and in peanuts (Sultan & Magan, 2011) have been demonstrated.

4. Emerging challenges in mycotoxin management

At present, the world is threatened by climate change and the potential dangers it brings. Climate change is an alteration of the average weather distributions over prolonged period of time (*i.e.*, decades to centuries) which is caused by several factors such as biotic processes, solar radiation and volcanic eruptions. The worldwide increase of atmospheric carbon dioxide (CO_2; second-most abundant greenhouse gas) and consequently the rise of temperature (*i.e.*, global warming) constitute the major manifestations of climate change. The average temperature of the lower troposphere (*i.e.*, lowest layer of Earth's atmosphere) has increased between 0.15 and 0.25°C per decade since 1980 according to satellite temperature measurements, while the atmospheric concentration of CO_2 has increased from 280 ppm in 1750 to 400 ppm in 2015 (ESRL, 2019). What is more alarming is that the increment trends of both parameters have been accelerating decade by decade, and are actually made worse by human activities. For instance, CO_2 has approximately increased from 280 ppm in

1750 (beginning of Industrial Revolution) to 330 ppm in 1970, which saw an increase of 50 ppm in around 220 years. However, the next 50 ppm steadily rose within just 30 years to 380 ppm (ca. 1970 – 2000), and the next 20 ppm in 15 years (ca. 2000 – 2015) to the present concentration of 411 ppm (ESRL, 2019). Worldwide, as a result of climate change, humans will face substantial threats in terms of health, food security due to decreasing crop yields, and the desertification of populated areas due to rising sea levels (Battisti & Naylor, 2009). Furthermore, there has also been an increasing concern that climate change permits for the increased propagation of crop pests and pathogens and their movement to other previously unsuitable regions (Bebber et al., 2013) due to the alteration in the natural environments which creates new opportunities for evolution (Fisher et al., 2012). The impacts of climate change on the propagation of microfungi, especially that of the mycotoxigenic fungi and the subsequent mycotoxin production, have been previously discussed in depth (Ksenija, 2018; Medina et al., 2017b; 2015; Botana & Sainz, 2015; Paterson & Lima, 2011; 2010).

As if the threats of climate change are not severe enough, we are also burdened by the recently discovered "masked mycotoxins". Masked mycotoxins are biologically modified mycotoxins which occur as a result of plant defense mechanism against xenobiotic compounds that alters the chemical structure of the mycotoxins through conjugation with sugars, amino acids or sulfate groups, and compartmentalize them in plant cell vacuoles or conjugated to biopolymers such as cell wall components. These conjugated mycotoxins are non-toxic. However, they could be converted back into their parent form by hydrolysis during food and feed processing or in the digestive tract of humans and animals (Berthiller et al., 2009a; 2016; Galaverna et al., 2009). The issue of masked mycotoxins began attracting scientific interest after several mysterious cases of mycotoxicoses during the mid-1980s, in which symptoms in severely affected animals did not correlate with the low mycotoxin content detected in their feed (Berthiller et al., 2009b; 2016). Furthermore, masked mycotoxins generally elude the established quantification analyses (*e.g.*, HPLC), thus leading to an underestimation of their exposure (hence the term "masked"), and masked mycotoxin standards, reference materials and inter-laboratory validated quantification methods are not yet commercially / widely available. The terms ''masked'', ''hidden'', ''conjugated'' (covalently-linked) and ''bound'' (non-covalently-linked) are frequently used in the literature, with masked being the most popular (Berthiller et al., 2009a). More recently, a new term, "modified mycotoxin", has been proposed which encompasses other possible forms of mycotoxins and their modifications including masked mycotoxins in the effort "to harmonize future scientific wording and subsequent legislation" (Rychlik et al., 2014).

The looming threats of climate change and its effects on the proliferation of mycotoxigenic fungi and the subsequent contamination of staple food and feed chains with mycotoxins, and the emergence of masked mycotoxins are seen as new global challenges that further impact on the management strategies for mycotoxins.

5. Conclusions and the way forward

As part of a good IPM, biological control of phytopathogenic organisms has been widely applied in Malaysia with all the four main biological control strategies (*i.e.*, *natural*, *importation*, *augmentation*, *conservation*) have been documented. The role of natural enemies in controlling native pests has been recognized in economically important crops especially oil palm, cocoa, coconut and rice. However, the same is not

entirely true for mycotoxigenic fungi, against which the application of biological control is almost non-existent. Based on the severely limited data, we conclude that this could either be due to the fact that (i) this particular application is totally new in Malaysia and is not yet recognized as an important research area, (ii) ongoing trials are not yet conclusive and published, or (iii) the safety consideration and risk analysis on whether or not to introduce biological control in Malaysian agro-ecosystem is taking longer than anticipated. This is rather perplexing since (i) Malaysian tropical climate is conducive for fungal proliferation including that of the mycotoxigenic fungi, (ii) reports on mycotoxin contamination of Malaysian foods and crop commodities are abound, (iii) Malaysia does have legislations for mycotoxin contamination, (iv) in other parts of the world, the application of biological control agents to combat mycotoxigenic fungi is more widely practiced and accepted, and (v) the application of biological control agents against plant diseases has long and successful history in Malaysia.

To fully utilize the abundant potentials of biological control approaches against mycotoxigenic fungi in the effort to minimize the impact and exposure of humans and animals to these toxic compounds, the followings are henceforth suggested:

- further and deeper investigation on the development and application of biological control against mycotoxigenic fungi and mycotoxin contamination using indigenous isolates
- as most studies have focused on the use of effective biological control agents against plant diseases, it may be beneficial to also screen these for mycotoxin control
- since fungal growth and mycotoxin production are not always parallel, care and consideration when selecting and developing a biological control agent should be taken to avoid mycotoxin stimulation (instead of mycotoxin inhibition)

CONFLICT OF INTEREST

None.

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AUTHORS CONTRIBUTIONS

Yazid, S.N.E. was involved in drafting the entire work. Selamat, J., Magan, N. and Samsudin, N.I.P. were involved in critically revising the “mycotoxigenic fungi” contents. Ismail, S.I. was involved in critically revising the “phytopathogenic organisms” contents.

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LIST OF TABLES

Table 1: Available publications on the use of biological control agents against phytopathogenic organisms in Malaysia (1980 – 2019). Publications were obtained based on a literature search on SCOPUS®. Search keywords: biological control > Malaysia. Biological control agents are sorted according to types of organisms.

Biological Control Agent	Targeted Pest / Pathogen / Disease	Application / Potential Mechanism of Action	Reference
(a) ASCOMYCETE			
Beauveria bassiana	*Atteva sciodoxa* (tiger moth) whose larvae infest long jack	Spraying of conidial suspension targeting larvae	Abood et al., 2010
Beauveria bassiana, *Paecilomyces fumosoroseus*	*Plutella xylostella* (diamondback moth) whose larvae infest cabbages	Spraying of conidial suspension	Ibrahim & Low, 1993
Exserohilum longirostratum	*Echinochloa crus-galli* (barnyard grass), weed infesting rice paddy fields	Spraying of conidial suspension	Ng et al., 2012
Exserohilum monoceras	*Echinochloa crus-galli* (barnyard grass), weed infesting rice paddy fields	Spraying of conidial suspension	Kadir et al., 2008
Exserohilum prolatum	*Rottboellia cochinchinensis* (itch grass), a noxious weed impacting crops	Soil treatment (spraying) with conidial suspension	Alloub et al., 2009
Exserohilum rostratum	*Cyperus iria* (flat sedge), weed infesting rice paddy fields	Spraying of conidial suspension	Kundat et al., 2010

Fusarium oxysporum (non-pathogenic)	*Fusarium oxysporum* f. sp. *cubense* (ascomycete) causing *Fusarium* wilt in bananas	As an endophyte	Ting et al., 2009
Isaria fumosorosea	*Coptotermes curvignathus*, *C. gestroi* (termites) infesting trees	Conidia germinate and penetrate into the termite cuticle	Jessica et al., 2019
Isaria fumosorosea, Metarhizium anisopliae	*Atteva sciodoxa* (tiger moth) whose larvae infest long jack	Spraying of conidial suspension	Sajap et al., 2014
Metarhizium anisopliae var. *anisopliae*	*Coptotermes curvignathus* (termite) infesting oil palms	Conidia germinate and penetrate into the termite cuticle	Hoe et al., 2009
Metarhizium anisopliae var. *major*	*Oryctes rhinoceros* (rhinoceros beetle) attacking oil palm fronds	Granules of mycelia and spores attacking beetle larvae	Moslim et al., 2011b; Moslim et al., 2009
Paecilomyces carneus, *P. farinosus*	*Pteroma pendula* (bagworm moth) whose larvae infest oil palms	Spraying of conidial suspension targeting larvae	Bakeri et al., 2009
Paecilomyces lilacinus	*Meloidogyne incognita* (nematode) causing root-knot in black peppers	Spraying of conidial suspension	Pau et al., 2012
Trichoderma harzianum	*Fusarium proliferatum*, *F. verticillioides* (ascomycete) causing *Fusarium* ear rot in maize	Production of volatile and non-volatile inhibitory compounds	Suhaida & NurAinIzzati, 2013
Trichoderma harzianum	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Production of volatile and non-volatile inhibitory compounds	Siddiquee et al., 2009; Nur Ain Izzati & Abdullah, 2008; Sundram et al., 2008
Trichoderma harzianum	*Macrophomina phaseolina* (ascomycete) causing charcoal rot disease in soybeans	Production of volatile and non-volatile inhibitory compounds	Khalili et al., 2016

Trichoderma virens	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Production of volatile and non-volatile inhibitory compounds	Angel et al., 2016
Trichoderma sp.	*Botryodiploidia theobromae* (ascomycete) causing stem-end rot in mangos	Fruit treatment (spraying) with conidial suspension	Suhanna et al., 2013
Trichoderma spp.	*Fusarium fujikuroi* (ascomycete) causing bakanae disease in rice	Production of volatile and non-volatile inhibitory compounds	Ng et al., 2015
Zoophthora radicans	*Plutella xylostella* (diamondback moth) whose larvae infest cabbages	Spraying of conidial suspension	Furlong et al., 1995
(b) BASIDIOMYCETE			
Clitopilus prunulus, *Grammothele fuligo*, *Lentinus tigrinus*, *Pycnoporus sanguineus*, *Trametes lactinea*	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Root treatment of oil palm seedlings	Naidu et al., 2018
Ganoderma orbiforme, *Neonothopanus nambi*, *Schizophyllum commune*	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Competition for substrate and space, and production of non-volatile inhibitory compounds	Naidu et al., 2016
Schizophyllum commune	Wood-degrading fungi on rubber wood	Production of inhibitory compounds	Teoh et al., 2017 Peng & Don, 2013; Teoh et al., 2012
(c) YEAST			
110 species were screened, of which 29 species showed antagonism	*Colletotrichum gloeosporioides* (ascomycete) causing anthracnose in papayas	Production of hydrolytic enzymes and antimicrobial compounds	Hamizah et al., 2013
(d) BACTERIUM			

Actinomycetes	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Production of inhibitory compounds	Shariffah-Muzaimah et al., 2018; Nur Azura et al., 2016; Shariffah-Muzaimah et al., 2015; Tan et al., 2002
Bacillus amyloliquefaciens, *Pseudomonas pachastrellae*	*Phytophthora capsici* (oomycete), *Fusarium solani* (ascomycete) infesting black peppers	Production of extracellular hydrolytic enzymes	Kota et al., 2015
Bacillus thuringiensis subsp. *Kurstaki*	*Metisa plana* (bagworm moth) whose larvae infest oil palms	Soil treatment (spraying) with bacterial suspension	Salim et al., 2015
Bacillus spp., *Enterobacter* spp.	*Fusarium solani* f. sp. *piperis*, *F. oxysporum* f. sp. *piperis* (ascomycetes) causing foot rot and stem blight in black peppers	Production of volatile and diffusible bioactive antifungal compounds	Edward et al., 2013
Bacillus spp., *Burkholderia* spp., *Pseudomonas* spp.	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	As an endophyte	Ramli et al., 2016 Seung et al., 2015;
Burkholderia cepacia	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Production of proteolytic enzymes	Azadeh et al., 2010
Burkholderia cepacia, *Pseudomonas aeruginosa*	*Colletotrichum gloeosporioides* (ascomycete) causing anthracnose in papayas	Production of volatile and non-volatile inhibitory compounds	Rahman et al., 2007

Burkholderia sp.	*Phytophthora capsici* (oomycete) causing foot rot and stem blight in black peppers	Production of hydrolytic enzymes and antimicrobial compounds	Ahmad & Ahmadu, 2017
Burkholderia sp., *Pseudomonas* sp.	*Fusarium oxysporum* f. sp. *cubense* (ascomycete) causing *Fusarium* wilt in bananas	As an endophytes which induces host defense enzymes	Mohd Fishal et al., 2010
Enterobacter asburiae, E. cancerogenus, E. cloacae	*Phytophthora capsici* (oomycete) causing foot rot and stem blight in black peppers	Production of volatile inhibitory compounds	Toh et al., 2016
Lactic acid bacteria	*Colletotrichum capsici* (ascomycete) causing anthracnose in chili	Seed treatment of chili	El-Mabrok et al., 2012
Pseudomonas aeruginosa	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	As an endophyte	Sathyapriya et al., 2012; Bivi et al., 2010
Pseudomonas fluorescens	*Fusarium oxysporum* f. sp. *cubense* (ascomycete) causing Fusarium wilt in bananas	Production of inhibitory compounds	Mohammed et al., 2011
Pseudomonas spp.	*Rhizoctonia solani* (basidiomycete) causing sheath blight of rice	Soil treatment (spraying) with bacterial suspension	Akter et al., 2016; Akter et al., 2014
Serratia marcescens	*Fusarium oxysporum* f. sp. *cubense* (ascomycete) causing *Fusarium* wilt in bananas	Soil treatment with inoculum which induces host defense enzymes	Ting et al., 2010
Streptomyces glauciniger	Phytopathogenic fungi	Production of chitinase	Awad et al., 2014

Streptomyces griseus	*Fusarium oxysporum* f. sp. *cubense* (ascomycete) causing *Fusarium* wilt in bananas	Soil treatment (spraying) with bacterial suspension	Zacky & Ting, 2015; Zacky & Ting, 2013
Streptomyces violaceusniger	*Fusarium oxysporum* f. sp. *cubense* (ascomycete) causing *Fusarium* wilt in bananas	Production of inhibitory compounds	Getha & Vikineswary, 2002
Streptomyces spp.	*Colletotrichum capsici*, *C. acutatum*, *C. gloeosporioides* (ascomycete) causing anthracnose in chili	Soil treatment (spraying) with bacterial suspension	Shahbazi et al., 2014; Shahbazi et al., 2013
Streptomyces spp.	*Pyricularia oryzae* (rice blast fungus) infesting rice	Production of inhibitory compounds	Awla et al., 2017; Law et al., 2017
(e) INSECT			
Acerophagus papayae (wasp)	*Paracoccus marginatus* (papaya mealybug) infesting papayas	As an endoparasitoid	Mastoi et al., 2018
Apanteles metesae (wasp)	*Metisa plana* (bagworm moth) whose larvae infest oil palms	As a parasitoid	Salmah et al., 2012
Calycomyza lantanae, *Ophiomyia lantanae* (flies)	*Lantana camara* (big sage), invasive weed	Larvae of flies are leaf-eaters	Ooi, 1987
Cotesia vestalis (wasp)	*Plutella xylostella* (diamondback moth) whose larvae infest cabbages	As a parasitoid	Kermani et al., 2014
Cosmolestes picticeps, *Sycanus dichotomus* (bugs)	*Metisa plana* (bagworm moth) whose larvae infest oil palms	As predatory natural enemies	Jamian et al., 2017

Diachasmimorpha longicaudata, *Fopius arisanus*, *F. vandenboschi*, *Psyttalia fletcheri*, *P. incisi* (wasps)	*Bactrocera carambolae, B. papayae, B cucurbitae* (fruit flies) infesting fruits	As a parasitoid	Yaakop et al., 2015; Shariff et al., 2014; Ibrahim et al., 2013; Chinajariyawong et al., 2000
Dolichoderus thoracicus (ant)	*Conopomorpha cramerella* (cocoa pod borer moth) whose larvae infest cocoa	Active and dispersive predatory behavior of ants	Adnan et al., 2018
Dolichoderus thoracicus, *Oecophylla smaragdina* (ants)	*Helopeltis theobromae* (mosquito bug) causing leaf tattering and fruit blemishes in cocoa	Active and dispersive predatory behavior of ants	Way & Khoo, 1991
Menochilus sexmaculatus (ladybird beetle)	*Rhopalosiphum maidis* (aphid) infesting maize	Predatory behavior of beetles	Ibrahim & Kueh, 2013
Micromus tasmaniae (lacewings)	Aphids infesting potatoes	Plant treatment (spraying) with egg suspension	Hussein, 1984
Oecophylla smaragdina (weaver ant)	*Hypsipyla robusta* (moth) whose larvae feed on timber shoots, flowers and barks	Active and dispersive predatory behavior of ants	Lim et al., 2008
Oecophylla smaragdina (weaver ant)	*Pteroma pendula* (bagworm moth) whose larvae infest oil palms	Active and dispersive predatory behavior of ants	Pierre & Idris, 2013
Tamarixia radiata (wasp)	*Diaphorina citri* (Asian citrus psyllid) infesting citrus fruits	As a parasitoid	Sule et al., 2014
(f) MICROSPORIDIA			

Nosema bombycis	*Plutella xylostella* (diamondback moth), *Spodoptera exigua* (beet armyworm moth) and *S. litura* (tobacco cutworm moth or cotton leafworm moth) whose larvae infest fruits and vegetables	Spraying of spore suspension targeting larvae	Ghani et al., 2013; Kermani et al., 2013; Ramli et al., 2011
(g) SHRUB			
Tinospora tuberculate	*Echinochloa crus-galli* (barnyard grass), weed infesting rice paddy fields	Production of volatile inhibitory compounds	Aslani et al., 2015
(h) SNAIL			
Pomacea canaliculata, *P. maculata*	*Limnocharis flava* (yellow velvetleaf), weed infesting rice paddy fields	As herbivore	Gilal et al., 2016
(i) VIRUS			
Baculovirus	*Spodoptera litura* (tobacco cutworm moth or cotton leafworm moth) whose larvae infest starfruits and vegetables	Spraying of viral occlusion bodies	Zabedah et al., 2010
Baculovirus	*Plutella xylostella* (diamondback moth) whose larvae infest cabbages	Spraying of viral occlusion bodies	Abdul Kadir et al., 1999
Cucumber Mosaic Virus	*Bemisia tabaci* (silverleaf whitefly) infesting chili	Induction of host resistance through artificial viral inoculation onto the plant	Saad et al., 2019
Nudivirus	*Oryctes rhinoceros* (rhinoceros beetle) infesting oil palm fronds	Spraying of viral occlusion bodies	Moslim et al., 2011a; Ramle et al., 2005
(j) COMBINATION			

A consortium of functional microbiota in microbial-enriched compost tea	*Golovinomyces cichoracearum* (ascomycete) causing powdery mildew on melons	Spraying of melon seedlings with the compost tea	Naidu et al., 2012
Bacillus thuringiensis (bacteria) *Metarhizium anisopliae* (ascomycete)	*Tirathaba rufivena* (bunch moth) whose larvae infest oil palms	Spraying with fungal and bacterial suspension	Mohamad et al., 2017
Bacillus spp. (bacteria) *Trichoderma* spp. (ascomycetes)	*Ganoderma boninense* (basidiomycete) causing basal stem rot in oil palms	Production of volatile and non-volatile inhibitory compounds	Angel et al., 2018; Alexander et al., 2017
Bacillus subtilis (bacterium) *Trichoderma* spp. (ascomycetes)	*Rhizoctonia solani* (basidiomycete) causing various commercially significant plant diseases	Soil treatment (spraying) with fungal and bacterial suspension	Ali & Nadarajah, 2013
Cataenococcus hispidus (mealybug) *Dolichoderus thoracicus* (ant)	*Helopeltis theobromae* (mosquito bug) causing leaf tattering and fruit blemishes in cocoa	Active and dispersive predatory behavior of ant and bug	Ho & Khoo, 1997
Eurytoma attiva (wasp) *Metrogaleruca obscura* (beetle)	*Cordia curassavica* (tropical black sage or wild sage), invasive weed	Defoliation by beetle and seed-destruction by wasp	Simmonds, 1980
Pseudomonas aeruginosa (bacterium) *Trichoderma harzianum, T. virens* (ascomycetes)	*Colletotrichum truncatum* (ascomycete) causing damping-off in soybeans	Seed treatment with conidial suspension	Begum et al., 2010

Table 2: The International Agency for Research on Cancer (IARC) Monographs evaluations of the carcinogenic hazard of selected mycotoxins. Adapted from Ostry et al. (2017). *Group 1: carcinogenic to humans (sufficient evidence), Group 2A: probably carcinogenic to humans (limited evidence of carcinogenicity in humans but sufficient evidence in experimental animals), Group 2B: possibly carcinogenic to humans (limited evidence of carcinogenicity in humans and less than sufficient evidence in experimental animals), Group 3: not classifiable as to its carcinogenicity to humans (evidence is inadequate in humans and inadequate or limited in experimental animals), Group 4: probably not carcinogenic to humans (evidence suggesting lack of carcinogenicity in humans and in experimental animals). At present, no known mycotoxin has been classified in groups 2A and 4. The complete listing of the LD_{50} of each mycotoxin can be obtained from the Toxicology Data Network, National Library of Medicine, National Institutes of Health, USA (https://toxnet.nlm.nih.gov/). LD_{50} = median lethal dose / semi-lethal dose / sublethal dose, which is the dose required to kill half the members of a tested population after a specified test duration, and is frequently used as a general indicator of a substance's acute toxicity.*

Mycotoxin	**Group**	**Reference**
Aflatoxins B_1, B_2, G_1, G_2, M_1	1	IARC (2012)
Citrinin	3	IARC (1987)
Cyclochlorotine	3	IARC (1987)
Deoxynivalenol	3	IARC (1993)
Fumonisin B_1	2B	IARC (2002)
Fumonisin B_2	2B	IARC (1993)
Fusarenone X	3	IARC (1993)
Fusarin C	2B	IARC (1993)
Kojic acid	3	IARC (2001)
Luteoskyrin	3	IARC (1987)
Nivalenol	3	IARC (1993)
Ochratoxin A	2B	IARC (1993)
Patulin	3	IARC (1987)
Penicillic acid	3	IARC (1987)
Rugulosin	3	IARC (1987)
Sterigmatocystin	2B	IARC (1987)
T-2 toxin	3	IARC (1993)
Zearalenone	3	IARC (1993)

Table 3: Maximum permitted proportion (µg/kg or ppb) of mycotoxins in foods. Adapted from Malaysian Food Regulations (1985).

FOOD	MAXIMUM PERMITTED PROPORTION (µg/kg or ppb)		
AFLATOXINS	**Total**	**B_1**	**M_1**
Groundnuts, almonds, hazelnuts, pistachios, Brazil nuts (shelled, for further processing)	15		
Groundnuts, almonds, hazelnuts, pistachios, Brazil nuts (shelled, ready-to-eat)	10		
Cereal-based food for infants and children		0.1	
Milk			0.5
Infant formula and follow-up formula (ready-to-drink)			0.025
Others	5		
OCHRATOXIN A			
Cereal-based food for infants and children	0.5		
Coffee or ground coffee or coffee powder	5		
Instant coffee or soluble coffee, decaffeinated coffee	10		
PATULIN			
Apple juice (includes apple juices as ingredients in other beverages)	50		

Table 4: Occurrence of mycotoxins on Malaysian food and feed.

Food / Feed (number of sample, *n*)	**Mycotoxin**	***n* positive, *n* above limit**	**Reference**

Spices and spice-based products (*n* = 90)	Aflatoxins, ochratoxin A	AFs *n* positive 19/90; *n* above limit 19/90 OTA *n* positive 37/90; *n* above limit 25/90	Ahmad-Zaidi et al., 2020
Commercial food and beverage (*n* = 120); apple, grape, orange, and pomegranate juices; wheat and barley flour; dried figs, raisins, chili powder, and spices; non-roasted peanut; roasted pistachio	Aflatoxins, ochratoxin A	AFs *n* positive 19/120; *n* above limit 14/120 OTA *n* positive 3/120; *n* above limit *none*	Alsharif et al., 2019
Raw peanuts (*n* = 87), peanut-based products (*n* = 91)	Aflatoxins	raw peanuts *n* positive 36/87; *n* above limit 20/87 peanut-based products *n* positive 50/91; *n* above limit 9/91	Norlia et al., 2018
Milk and dairy products (*n* = 53)	Aflatoxin M_1	*n* positive 19/53; *n* above limit 4/53	Nadira et al., 2017
Cow's, goat's, human's milk (*n* = 33)	Aflatoxin M_1	*n* positive 2/33; *n* above limit *none*	Shuib et al., 2017a
Cow's milk (*n* = 102)	Aflatoxin M_1	*n* positive 4/102; *n* above limit 3/102	Shuib et al., 2017b
Commercial vegetable oil (*n* = 25)	Aflatoxins, ochratoxin A, deoxynivalenol, zearalenone	AFs *n* positive *none*; *n* above limit *none* OTA *n* positive *none*; *n* above limit *none* DON *n* positive *none*; *n* above limit *none* ZEN *n* positive 15/25; *n* above limit *none*	Sharmili et al., 2016

Commercial spice (*n* = 58)	Aflatoxins, ochratoxin A	AFs *n* positive 50/58; *n* above limit 15/58 OTA *n* positive 27/34; *n* above limit 1/34	Ali et al., 2015
Red yeast rice (traditional Chinese medicine; *n* = 50)	Aflatoxins, ochratoxin A, citrinin	AFs *n* positive 46/50; *n* above limit 35/50 OTA *n* positive 50/50; *n* above limit *none* CIT *n* positive 50/50; *n* above limit 50/50	Samsudin and Abdullah, 2013
Commercial dried chili (*n* = 80)	Aflatoxins, ochratoxin A	AFs *n* positive 52/80; *n* above limit 9/80 OTA *n* positive 65/80; *n* above limit 13/80	Jalili and Jinap, 2012
Commercial rice (*n* = 5), peanut (*n* = 9) and chili (*n* = 10)	Aflatoxins	rice *n* positive 4/5; *n* above limit 3/5 peanut *n* positive 4/9; *n* above limit 2/9 chili *n* positive 9/10; *n* above limit 3/10	Khayoon et al., 2012
Commercial coffee and cereal (*n* = 45)	Ochratoxin A	*n* positive 25/45; *n* above limit 1/45	Lee et al., 2012
Commercial cereal (*n* = 100)	Aflatoxins B_1, B_2, G_1, G_2, ochratoxin A, zearalenone, deoxynivalenol, fumonisins B_1, B_2, T-2, HT-2	AFs *n* positive 60/100; *n* above limit 2/100 OTA *n* positive 40/100; *n* above limit 2/100 ZEN *n* positive 25/100; *n* above limit *none*	Soleimany et al., 2012a

		DON *n* positive 36/100; *n* above limit *none* FUM *n* positive 19/100; *n* above limit *none* T-2 *n* positive 16/100; *n* above limit *none* HT-2 *n* positive 16/100; *n* above limit *none*	
Commercial cereal (*n* = 80)	Aflatoxins B_1, B_2, G_1, G_2, ochratoxin A, zearalenone, deoxynivalenol, fumonisins B_1, B_2, T-2, HT-2	AFs *n* positive 40/80; *n* above limit 2/80 OTA *n* positive 24/80; *n* above limit 1/80 ZEN *n* positive 15/80; *n* above limit *none* DON *n* positive 24/80; *n* above limit *none* FUM *n* positive 13/80; *n* above limit *none* T-2 *n* positive 11/80; *n* above limit *none* HT-2 *n* positive 9/80; *n* above limit *none*	Soleimany et al., 2012b
Commercial food products (*n* = 95)	Aflatoxin B_1	*n* positive 69/95; *n* above limit *none*	Reddy et al., 2011
Grain corn for animal feed (*n* = 80)	Aflatoxin B_1, fumonisins	AFB_1 *n* positive 65/80; *n* above limit 18/80 FUM *n* positive 80/80;	Reddy & Salleh, 2011

		n above limit *none*	
Commercial food and feed (*n* = 39)	Fumonisins	*n* positive 5/39; *n* above limit *none*	Khayoon et al., 2010a
Animal feed (*n* = 42)	Aflatoxins	*n* positive 8/42; *n* above limit 3/42	Khayoon et al., 2010b
Commercial wheat (*n* = 15) and barley (*n* = 15)	Aflatoxin B_1	wheat *n* positive 3/15; *n* above limit *none* barley *n* positive 1/15; *n* above limit	Reddy & Salleh, 2010
Commercial cereal (*n* = 60)	Aflatoxins, ochratoxin A, zearalenone	AFs *n* positive 24/60; *n* above limit 1/42 OTA *n* positive 9/60; *n* above limit 2/42 ZEN *n* positive 11/60; *n* above limit *none*	Rahmani et al., 2010
Commercial noodle (*n* = 135)	Deoxynivalenol	*n* positive 110/135; *n* above limit *none*	Moazami & Jinap, 2009
Raw shelled peanut (*n* = 145)	Aflatoxins	*n* positive 73/145; *n* above limit 33/145	Sulaiman et al., 2007

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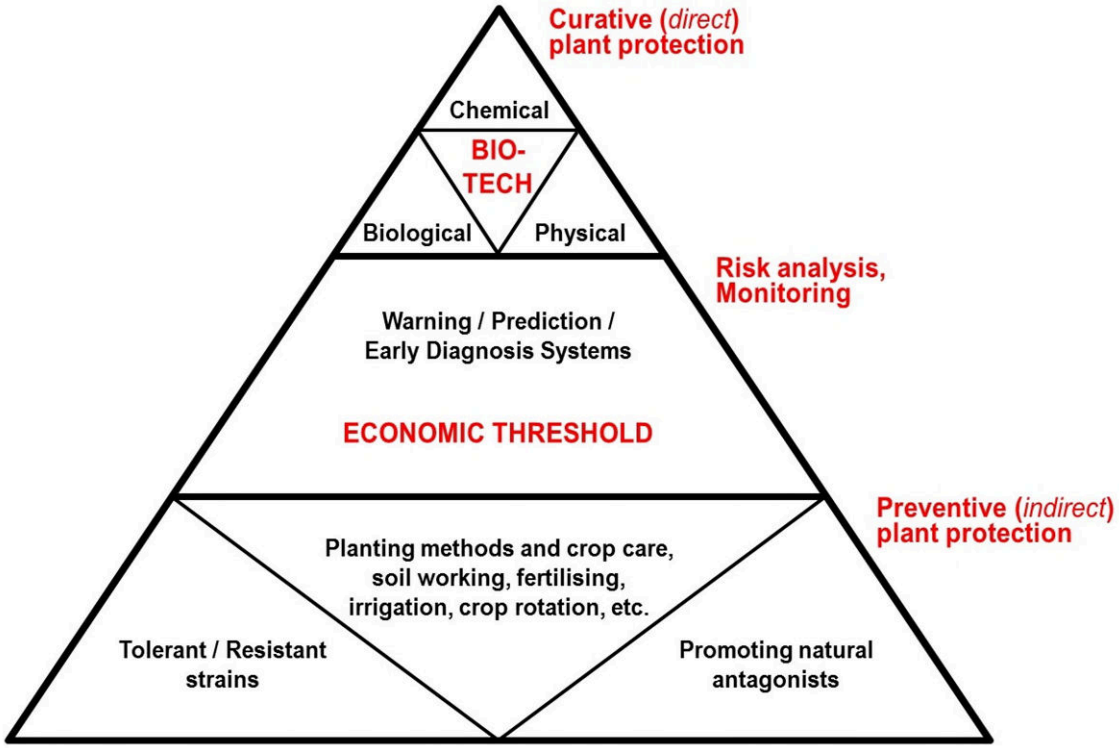


Figure 1: The principles and approaches of Integrated Plant Protection (IPP) otherwise known as Integrated Pest Management (IPM). Adapted from Frische et al. (2018).

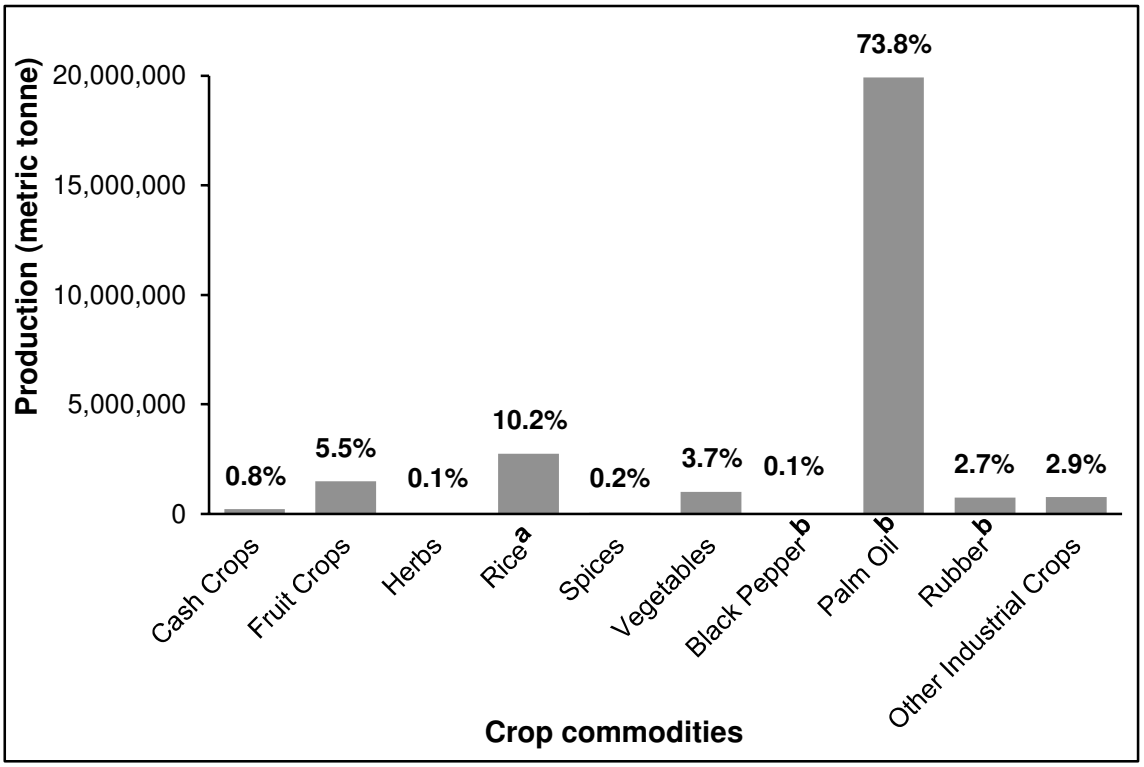


Figure 2: Malaysian agricultural production for the year 2017. Adapted from DOA (2019). [a]2015 data. [b]"Major industrial crops", as managed by the Malaysian Ministry of Primary Industries. Data for other "major industrial crops" such as timber, kenaf (*Hibiscus cannabinus*) and tobacco (*Nicotiana tabacum*) are not publicly available. "Other industrial crops" and other crop commodities are managed by the Malaysian Ministry of Agriculture and Agro-based Industry. "Cash crops" include cassava (*Manihot esculenta*), groundnut (*Arachis hypogeae*), maize (*Zea mays*), sugar cane (*Saccharum officinarum*), sweet potato (*Ipomoea batatas*), taro (*Colocasia esculenta*) and yam bean (*Pachyrhizus erosus*). "Other industrial crops" include areca nut (*Areca catechu*), coconut (*Cocos nucifera*), coffee (*Coffea arabica*), mushroom (various species), nipa palm (*Nypa fruticans*), roselle (*Hibiscus sabdariffa*), sago (*Metroxylon sagu*) and tea (*Camellia sinensis*).

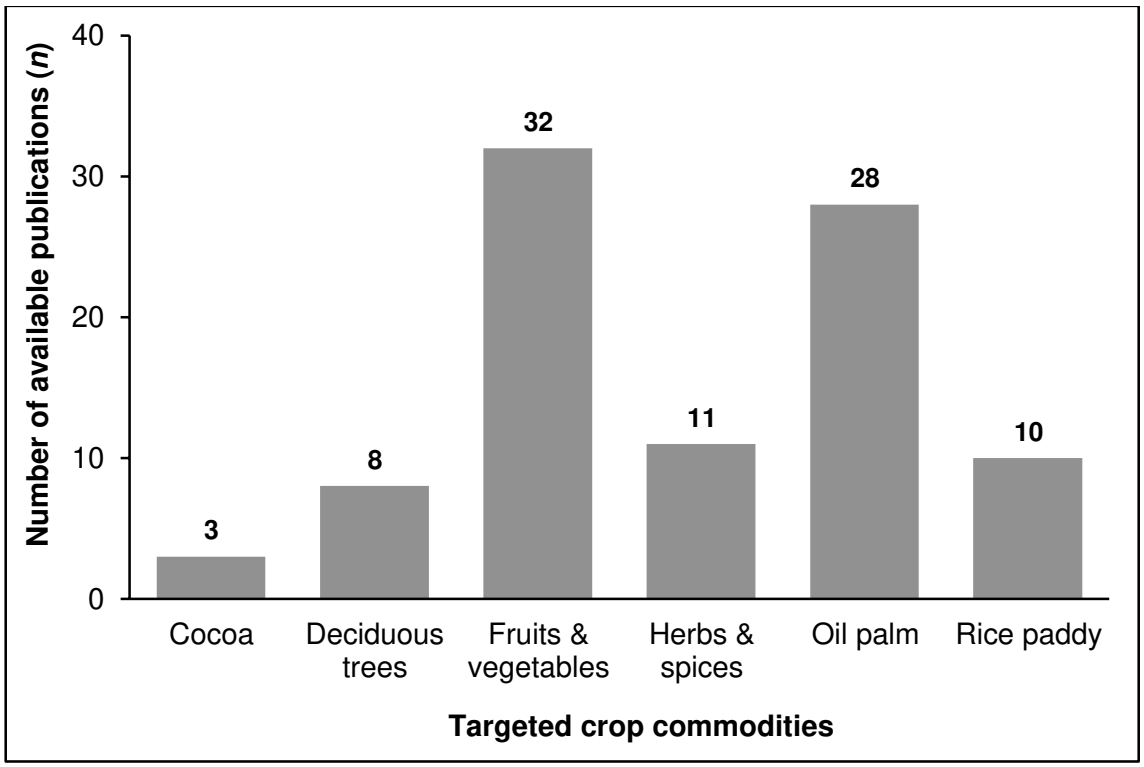


Figure 3: Distribution of targeted crop commodities on which scientific investigations on potential biological control agents against phytopathogenic organisms have been conducted in Malaysia (1980 – 2019). Publications were obtained based on a literature search on SCOPUS®, as similarly listed in Table 1. Search keywords: biological control > Malaysia.

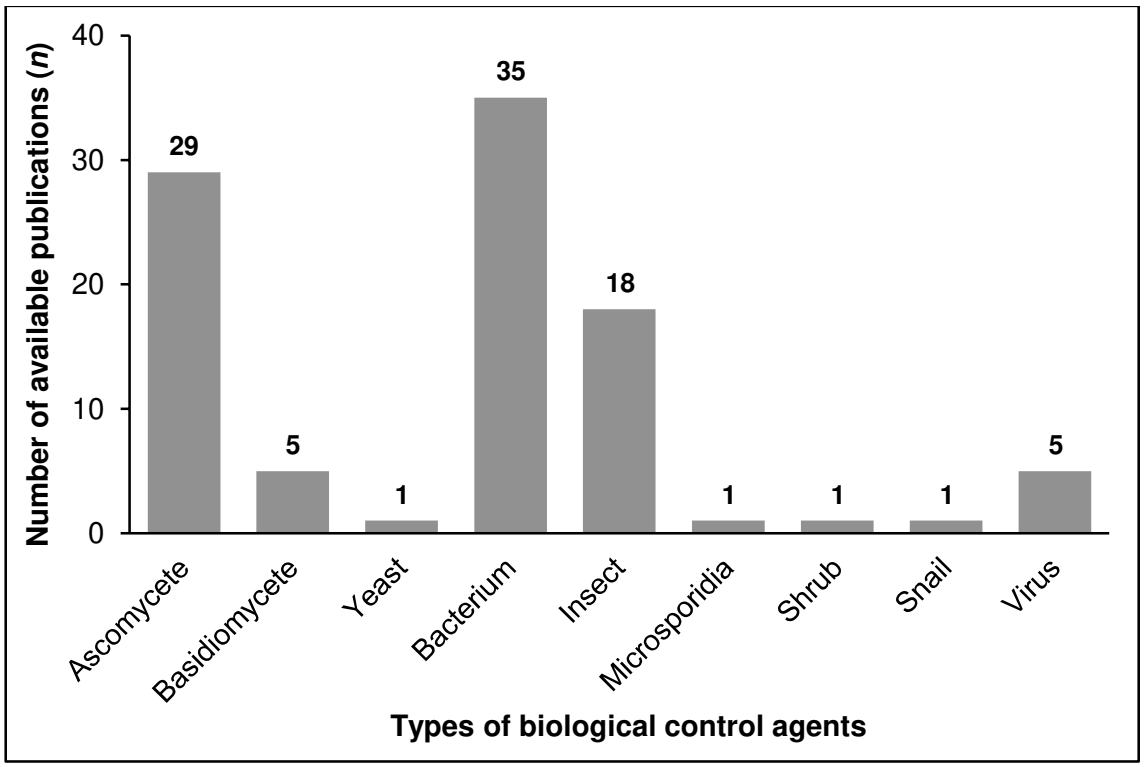


Figure 4: Number of available publications on potential biological control agents against phytopathogenic organisms from Malaysia (1980 – 2019). Publications were obtained based on a literature search on SCOPUS®, as similarly listed in Table 1. Search keywords: biological control > Malaysia.



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Phytopathogenic organisms and mycotoxigenic fungi: Why do we control one and neglect the other? A biological control perspective in Malaysia

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