Photosynthesis of Sago Palm (*Metroxylon sagu* Rottb.) Seedling at Different Air Temperatures
Current Status and Recent Developments in Biopesticide Use

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Received: 28 November 2017; Accepted: 8 January 2018; Published: 12 January 2018

Abstract: Biopesticides have attracted attention in pest management in recent decades, and have long been promoted as prospective alternatives to synthetic pesticides. Biopesticides have also attracted great interest in the international research community, with a significant increase in the number of publications devoted to the subject. Recently, new substances, like strains of the fungus Talaromyces flavus SAY-Y-94-01, extracts of the plant Clitoria ternatea (butterfly pea), products of the fungus Trichoderma harzianum, products of the bacterium Bacillus thuringiensis var. tenebrionis strain Xd3 (Btt-Xd3), the alkaloid compound oxymatrine, fermentation products of the bacterium Lactobacillus casei strain LPT-111, stilbenes accumulated in grape canes, and olive mill wastes, have been reported in the literature as promising compounds for use as biopesticides, but more field research is required to assess the effects on specific pest problems under diverse cropping systems. Nevertheless, biopesticides have not yet reached the desired level of use, whereby they could displace the dominance of chemical pesticides, given that the commercialization of new products in the market is lagging behind. Currently, biopesticides comprise a small share of the total crop protection market globally, with a value of about $3 billion worldwide, accounting for just 5% of the total crop protection market. Fewer biopesticide-active substances are registered in the European Union (EU) than in the United States, India, Brazil, or China, due to long and complex registration processes in the EU, which follow the model for the registration of conventional pesticides. Nanoformulations and microencapsulation technologies can improve the stability and residual action of biopesticide products, and this could increase their field use. Regulations that promote registration of low-risk compounds with the provision of incentives could also facilitate commercialization and availability of biopesticides in the market.

Keywords: botanical pesticides; market; semiochemicals; regulation

1. Introduction

Pesticide use has certainly contributed towards improving agricultural production, in terms of both yield and quality, thus increasing agricultural income, particularly in developed countries. However, careless use of pesticides without adhering to the safety norms and recommended practices has posed serious health risks to humans, other living organisms, and the environment, from on-farm workers’ exposure and release of chemicals into the air and water, to commodities containing pesticide residues [1,2]. Therefore, there has been a growing demand for food safety and quality in recent decades, as reflected in the tight safety regulations on imports of products and strict regulations on the amount of pesticide residues on commodities. Moreover, increasingly high standards regarding product quality are continuously being set. Public awareness about the adverse effects of pesticides on the safety of foods and on the environment has increased in recent years, and the search for alternatives to widely used chemical pesticides, including biopesticides, has become a priority. In this regard,
the conventional pesticide industry and market have undergone major changes over recent decades [3], which have entailed greater efficiency of pesticide use than in the past through major improvements to pest management technology and practices in the context of Integrated Pest Management (IPM) programs. These developments have significantly improved pest management practices, reduced, in some cases, pesticide use, and have also impeded the growth in demand for chemical pesticides [4].

2. Biopesticides Market

Biopesticides are natural materials derived from animals, plants, and bacteria, as well as certain minerals, that are used for pest control [5]. Almost 90% of the microbial biopesticides currently available on the market are derived from only one entomopathogenic bacterium, i.e., Bacillus thuringiensis or Bt [6]. Currently, biopesticides comprise a small share of the total crop protection market globally, with a value of about $3 billion worldwide, accounting for just 5% of the total crop protection market [7,8]. In the United States (US) market, more than 200 products are available, compared to 60 analogous products in the European Union (EU) market [6]. Although biopesticide use at a global scale is increasing by almost 10% every year [6], it appears that the global market must increase further in the future if these pesticides are to play a visible role in substituting for chemical pesticides and reducing the current over-reliance on them. It should be noted, however, that biopesticides are assessed in the EU by the same regulations used for the assessment of synthetic active substances, and this situation required several new provisions in the current legislation, as well as the preparation of new guidelines facilitating the registration of prospective biopesticide products [9]. Currently, there are fewer biopesticide active substances registered in the EU than in the US, India, Brazil, or China. The relatively low level of biopesticide research in the EU is related to the greater complexity of EU-based biopesticide regulations [10].

Growth of biopesticides is projected to outpace that of chemical pesticides, with compounded annual growth rates of more than 15% [7]. It is expected that biopesticides will equalize with synthetics, in terms of market size, between the late 2040s and the early 2050s, but major uncertainties in the rates of uptake, especially in areas like Africa and Southeast Asia, account for a major portion of the flexibility in those projections [8]. Biopesticides have been becoming increasingly popular in recent years, and are considered safer than conventional pesticides. As opposed to conventional pesticides, biopesticides are by their nature less detrimental and are more specific to the target pests. Additionally, biopesticides are effective in small amounts, and decompose quickly, without leaving problematic residues; therefore, they could reduce the use of conventional pesticides as integral components of IPM programs. It should be noted, however, that while case-specific exceptions to the above-mentioned properties may exist, they do not cancel the general rule. The development of the biopesticide market in the future is strongly related to research on biological control agents. Several scientists from diverse research institutes have done some preliminary research in the field, but complete and systematic reports are scarce. Therefore, it is necessary to strengthen the collaboration of enterprises and research institutes on this topic. It seems that biopesticides cannot as yet completely replace chemical pesticides, so the agricultural sector can and should benefit from the co-existence of biopesticides with chemical pesticides. In this regard, accelerating practical application of research results is expected to facilitate large-scale industrial development.

The pipeline of new chemistry has declined considerably in recent decades, as regulations have become tighter, with products being withdrawn from the market as they no longer meet the strict regulations. Therefore, a more limited choice of chemical solutions remains focused on many pests in few staple crops. These consequences, which have always been apparent in the pesticide market, are now more evident than ever.
3. New Substances

Recently, several new substances have been reported in the literature as promising compounds for use as biopesticides (Table 1), but more field research is necessary for assessing their efficacy on specific pest problems under diverse cropping systems.

| Product | Target Pest | Reference |
|---------|-------------|-----------|
| Strains of the fungus *Talaromyces flavus* SAY-Y-94-01 | Anthracnose caused by *Glomerella cingulata* and *Colletotrichum acutatum* | Ishikawa [11] |
| Extract of the species *Clitoria ternatea* (butterfly pea) | Helicoverpa spp. | Mensah et al. [12] |
| Products of the fungus *Trichoderma harzianum* | Fusarium root rot | Kirk and Schafer [13] |
| *Bacillus thuringiensis* var. *tenebrionis* strain Xd3 (Btt-Xd3) | Alder leaf beetle (*Agelastica alni*) | Eski et al. [14] |
| Alkaloid compound oxymatrine | Spodoptera litura, Helicoverpa armigera, *Aphis gossypii* | Rao and Kumari [15] |
| Fermentation products of the bacterium *Lactobacillus casei* strain LPT-111 | Angular leaf spot caused by *Xanthomonas fragariae* | Dubois et al. [16] |
| Stilbenes isolated from grapevine extracts | *S. littoralis* | Pavela et al. [17] |
| Olive mill waste | Various pests | El-Abbassi et al. [18] |

Strains of the fungus *Talaromyces flavus* were obtained from strawberry (*Fragaria* spp.) crowns for the purpose of checking for their inhibitory effect on anthracnose caused by *Glomerella cingulata* and *Colletotrichum acutatum* in the nursery [11]. Strong suppression of the disease in pretreated plants with the suspension of the strains was noted for all 13 strains used, with the strain SAY-Y-94-01 showing the highest inhibitory effect, equivalent to the well-known fungicide propineb [11]. Furthermore, six fractions of an extract of the species *Clitoria ternatea* (butterfly pea) were evaluated for bioactivity against species of the genus *Helicoverpa*, showing a preventive activity in oviposition, feeding, and direct toxicity to *Helicoverpa* spp. [12]. A commercial product (Sero-X) with the fractions of *Clitoria ternatea* has been developed that is active on *Helicoverpa* spp. and sucking pests, and is at the stage of commercialization. The efficacy of new biological substances for the control of *Fusarium* root rot disease of gladiolus hybrids has been tested, with corm-applied products of the fungus *Trichoderma harzianum* being among the most effective treatments against *Fusarium* root rot [13]. In addition, twenty-one *Bacillus* isolates were screened for insecticidal effects on alder leaf beetle (*Agelastica alni*) larvae and adults [14]. *Bacillus thuringiensis* var. *tenebrionis* strain Xd3 (Btt-Xd3) showed the greatest effect, while for high sporulation of the strain Xd3 and production of a toxin protein, suitable conditions (e.g., medium, temperature, and pH) were determined. The alkaloid compound oxymatrine controlled *Spodoptera litura*, *Helicoverpa armigera*, and *Aphis gossypii* effectively, and was found to be safe to predators, i.e., *Coccinella* spp. and parasites, i.e., *Trichogramma* spp. indicating that the alkaloid could be safe for non-target organisms [15]. Moreover, fermentation products of the bacterium *Lactobacillus casei* strain LPT-111 under the name Tivano, a new organic acid-based biopesticide, were found to maintain suppression of angular leaf spot, caused by *Xanthomonas fragariae*, for the duration of the trial [16]. Stilbenes isolated from grapevine extracts did not show any significant antifeedant effect or acute toxicity, but they caused chronic mortality to the larvae population of an important pest, *S. littoralis* [17]. Olive mill waste contains compounds that could be used for pest control in the Mediterranean area, but more research in the field is required to assess effects on specific pest problems under diverse cropping systems and their potential use in organic systems [18].

4. Nanotechnology

Several studies have reported an enhancement in the efficacy of certain biological substances on pests, a decrease in toxicity towards humans and the environment, and a reduction of losses due to
physical degradation (e.g., volatilization and leaching) with the encapsulation of these substances in nanoparticulate systems [19–21]. Thus, nanotechnology could contribute to the development of less toxic biopesticides with favorable safety profiles and increased stability of the active agents, enhanced activity on target pests, and increased adoption by the end-users [22–24]. Research has shown that the use of nanoparticles is effective in protecting neem (Azadirachta indica) oil from rapid degradation, allowing a prolonged effect on target pests. Because the polymers used in this kind of formulation are biodegradable, continuous delivery of the active agent with low environmental harm is achieved. Future research must target ways of circumventing the risk factors associated with nanoparticle usage, because currently comprehensive knowledge of risk assessment factors and further toxicity of nanoparticles towards agroecosystem components after their release into the environment is lacking [25]. Overall, nanobiotechnology seems promising in the direction of formulations that can be used to improve the stability and effectiveness of natural products [26,27]. Such formulations can provide controlled release of the molecules at the site of action, can minimize potential toxic effects on non-target organisms, and can prevent degradation of the active agent by microorganisms [27–29].

While there is certainly industrial activity aimed in this direction, the technology is still far from proven, with major questions persisting around release rates, storage stability, and cost effectiveness.

5. Regulation of Biopesticides

Several stakeholders, including scientists, regulators, marketers, and end-users, are involved in the development and commercialization chain of pest control products. Some participants in this chain are often involved from the earliest stages of the development process, but there are many issues still to be resolved; the marketers may often disagree with the regulators and scientists, such that end-users are often puzzled by perceived weaknesses in the final product. Data requirements for biological products are usually acquired from those derived for synthetic chemicals. For biopesticides, however, the assessment of risk should be based on scientific evidence appropriate to the substance, and should not follow rules pertaining to synthetic chemicals. Therefore, an adaptation of the requirements tailored to the nature of the various categories used in biopesticide active substances is required. Currently, data requirements and guidance documents are being properly adapted for biopesticides [30]. Submission procedures both at EU and Member State levels are lengthy, which seems to be the most pressing problem for the biocontrol industry. Considering that if new products are able to reach the market quickly, they will generate income, then faster procedures and enforcement of time limits are important. Moreover, the high cost related to the registration of new agents is another aspect limiting the commercialization of new products [31]. It appears that the registration process of biopesticide products impedes the commercialization of these products. Therefore, the regulatory authorities should try to ensure fast-track registration of biopesticide products based on justified regulations, promoting the adoption of safer technologies in the development of commercial products. Additionally, the regulatory system should enable small and medium-sized firms dealing with biopesticides to develop, so that they can provide growers with reliable tools for the economical control of pests, and allow them to provide products that meet the expectations of consumers.

6. Future Prospects

Biopesticides have long been attracting global attention as a safer strategy than chemical pest control, with potentially less risk to humans and the environment. To this end, co-operation between the public and private sectors is required to facilitate the development, manufacturing, and sale of this environmentally friendly alternative. In this context, discovery of new substances and research on formulation and delivery would boost commercialization and use of biopesticides. Additional research on integrating biological agents into common production systems is necessary. Maintaining low cost to farmers for a given product quality and availability, particularly in developing countries, is also important. Moreover, regulations that promote registration of low-risk compounds with provision of
incentives could also facilitate commercialization and availability of biopesticides in the market. While new substances could serve as a promising option for use in pest control, more field research is required to assess the efficacy on specific pest problems in various cropping systems. Microencapsulation based on nanotechnology could improve the residual action of biopesticides, and this could increase their field use.

Author Contributions: Both authors contributed equally in the conception and development of this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Damalas, C.A. Understanding benefits and risks of pesticide use. *Sci. Res. Essays* 2009, 4, 945–949.
2. Carvalho, F.P. Pesticides, environment, and food safety. *Food Energy Secur.* 2017, 6, 48–60. [CrossRef]
3. Pelaez, V.; Mizukawa, G. Diversification strategies in the pesticide industry: From seeds to biopesticides. *Ciência Rural* 2017, 47, e20160007. [CrossRef]
4. Baesso, M.M.; Teixeira, M.M.; Ruas, R.A.A.; Baesso, R.C.E. Pesticide application technologies. *Rev. Ceres* 2014, 61, 780–785. [CrossRef]
5. EPA. Biopesticides. Available online: www.epa.gov/pesticides/biopesticides (accessed on 9 September 2017).
6. Kumar, S.; Singh, A. Biopesticides: Present status and the future prospects. *J. Fertil. Pestic.* 2015, 6, e129. [CrossRef]
7. Marrone, P.G. The market and potential for biopesticides. In *Biopesticides: State of the Art and Future Opportunities*; Gross, A.D., Coats, J.R., Duke, S.O., Seiber, J.N., Eds.; American Chemical Society: Washington, DC, USA, 2014; pp. 245–258.
8. Olson, S. An analysis of the biopesticide market now and where is going. *Outlooks Pest Manag.* 2015, 26, 203–206. [CrossRef]
9. Czaja, K.; Góralczyk, K.; Struciński, P.; Hernik, A.; Korcz, W.; Minorczyk, M.; Ludwicki, J.K. Biopesticides—Towards increased consumer safety in the European Union. *Pest Manag. Sci.* 2015, 71, 3–6. [CrossRef] [PubMed]
10. Balog, A.; Hartel, T.; Loxdale, H.D.; Wilson, K. Differences in the progress of the biopesticide revolution between the EU and other major crop-growing regions. *Pest Manag. Sci.* 2017, 73, 2203–2208. [CrossRef] [PubMed]
11. Ishikawa, S. Integrated disease management of strawberry anthracnose and development of a new biopesticide. *J. Gen. Plant Pathol.* 2013, 79, 441–443. [CrossRef]
12. Mensah, R.; Moore, C.; Watts, N.; Deseo, M.A.; Glennie, P.; Pitt, A. Discovery and development of a new semiochemical biopesticide for cotton pest management: Assessment of extract effects on the cotton pest *Helicoverpa* spp. *Entom. Exp. Appl.* 2014, 152, 1–15. [CrossRef]
13. Kirk, W.W.; Schafer, R.S. Efficacy of new active ingredient formulations and new biopesticides for managing Fusarium root rot disease of gladiolus hybrids. *Acta Hortic.* 2015, 1105, 55–60. [CrossRef]
14. Eski, A.; Demir, D.; Sezen, K.; Demirbağ, Z.A. New biopesticide from a local *Bacillus thuringiensis* var. *tenebrionis* (Xd3) against alder leaf beetle (Coleoptera: Chrysomelidae). *World J. Microbiol. Biotechnol.* 2017, 33, 95. [PubMed]
15. Rao, P.V.M.; Kumari, A. Effect of oxymatrine 0.5% EC on predators and parasites of important pests on certain vegetable crops cultivated in Ranga Reddy District (Telangana). *Pestology* 2016, 40, 15–18.
16. Dubois, C.; Arsenaught-Labrecque, G.; Pickford, J. Evaluation of a new biopesticide against angular leaf spot in a commercial operation system. *Acta Hortic.* 2017, 1156, 757–764. [CrossRef]
17. Pavela, R.; Wafio-Teguo, P.; Biais, B.; Richard, T.; Mérimon, J.-M. *Vitis vinifera* canes, a source of stilbenoids against *Spodoptera littoralis* larvae. *J. Pest Sci.* 2017, 90, 961–970. [CrossRef]
18. El-Abbassi, A.; Saadoua, N.; Kair, H.; Raiti, J.; Hafidi, A. Potential applications of olive mill wastewater as biopesticide for crops protection. *Sci. Total Environ.* 2017, 576, 10–21. [CrossRef] [PubMed]
19. De Oliveira, J.L.; Campos, E.V.R.; Bakshi, M.; Abhilash, P.C.; Fraceto, L.F. Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: Prospects and promises. *Biotechnol. Adv.* 2014, 32, 1550–1561. [CrossRef] [PubMed]
20. Bakry, A.M.; Abbas, S.; Ali, B.; Majeed, H.; Abouelwafa, M.Y.; Mousa, A.; Liang, L. Microencapsulation of oils: A comprehensive review of benefits, techniques, and applications. *Compr. Rev. Food Sci. Food Saf.* 2016, 15, 143–182. [CrossRef]
21. Giongo, A.M.M.; Vendramim, J.D.; Forim, M.R. Evaluation of neem-based nanoformulations as alternative to control fall armyworm. *Ciênc. Agrotec.* 2016, 40, 26–36. [CrossRef]
22. Khot, L.R.; Sankaran, S.; Maja, J.M.; Ehsani, R.; Schuster, E.W. Applications of nanomaterials in agricultural production and crop protection: A review. *Crop Prot.* 2012, 35, 64–70. [CrossRef]
23. Agrawal, S.; Rathore, P. Nanotechnology pros and cons to agriculture: A review. *Int. J. Curr. Microbiol. Appl. Sci.* 2014, 3, 43–55.
24. Prasad, R.; Kumar, V.; Prasad, K.S. Nanotechnology in sustainable agriculture: Present concerns and future aspects. *Afr. J. Biotechnol.* 2014, 13, 705–713.
25. Mishra, S.; Keswani, C.; Abhilash, P.C.; Fraceto, L.F.; Singh, H.B. Integrated approach of agri-nanotechnology: Challenges and future trends. *Front. Plant Sci.* 2017, 8, 471. [CrossRef] [PubMed]
26. Ghormade, V.; Deshpande, M.V.; Paknikar, K.M. Perspectives for nano-biotechnology enabled protection and nutrition of plants. *Biotechnol. Adv.* 2011, 29, 792–803. [CrossRef] [PubMed]
27. Perlatti, B.; de Souza Bergo, P.L.; Fernandes da Silva, M.F.; Fernandes, J.B.; Forim, M.R. Polymeric nanoparticle-based insecticides: A controlled release purpose for agrochemicals. In *Insecticides-Development of Safer and More Effective Technologies*; Trdan, S., Ed.; InTech: Rijeka, Croatia, 2013; pp. 523–550.
28. Durán, N.; Marcato, P.D. Nanobiotechnology perspectives. Role of nanotechnology in the food industry: A review. *Int. J. Food Sci. Technol.* 2013, 48, 1127–1134. [CrossRef]
29. Gogos, A.; Knauer, K.; Bucheli, T.D. Nanomaterials in plant protection and fertilization: Current state, foreseen applications, and research priorities. *J. Agric. Food Chem.* 2012, 60, 9781–9792. [CrossRef] [PubMed]
30. Isman, M.B. A renaissance for botanical insecticides? *Pest Manag. Sci.* 2015, 71, 1587–1590. [CrossRef] [PubMed]
31. Pavela, R. Limitation of plant biopesticides. In *Advances in Plant Biopesticides*; Singh, D., Ed.; Springer Publishing: New Delhi, India, 2014; pp. 347–359.

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