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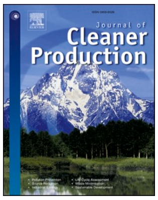

Analysis of yellow mealworm (*Tenebrio molitor*) frass as a resource for a sustainable agriculture in the current context of insect farming industry growth

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ABSTRACT

With the ongoing rapid growth of the human population, the industrial development of mass insect rearing for feed and food is gaining momentum. This approach has proved to be a more efficient and effective alternative to traditional livestock to produce animal protein. In this context, the *Tenebrio molitor* production sector emerges as highly promising in the edible insect market, being among the most commonly bred species in the large-scale insect rearing industry. A recent modification of the European regulatory framework in this matter has authorized insect-produced feed for pigs and poultry, which will boost the creation of new insect industries, resulting in huge quantities of frass, the insect excrement, that could be valorized. This by-product holds substantial amounts of nutrients and advantageous microorganisms that have enormous potential for organic farming. It typically exhibits high organic content and an abundance of essential nutrients like nitrogen (N), phosphorus (P), and potassium (K), alongside a narrow carbon (C) to nitrogen (N) ratio. In addition to providing plants with essential macronutrients, frass has been discovered to boost soil microbial biomass and introduce biomolecules and microbes that foster plant growth. The study of utilizing frass in sustainable agriculture is a relatively new and actively researched field in recent years. By conducting a comprehensive review of peer reviewed works, this article highlights the advantages associated with the effects of its use as organic fertilizer. Among these, the effect of frass as (1) a plant growth promoter and productivity enhancer and as (2) phytofortifyer against biotic and abiotic stresses, has been underlined in recent studies. With regard to its promoting effect, it has been observed that the effects of frass depend very much on its dose and the type of crop, with remarkably favorable results in many of the tests carried out. Hence, frass derived from the mass rearing of yellow mealworm for feed and food, could serve as an important and potent source of organic fertilizer in intensive horticultural crops or in high-value woody crops, contributing to a more sustainable agriculture, which can decrease the use of conventional synthetic agrochemicals.

1. Introduction

The world's population is growing steadily year after year with the rate of human population growth surpassing projections (O'Sullivan, 2023). In the year 2022, the world's population reached 8 billion people, starting from a population of 5 billion in 1986 or 7.7 billion in year 2019 (Gu et al., 2021; Taseska et al., 2023). This number will continue to grow rapidly in the coming decades, as it is estimated that by 2050 the population will reach peaks of 10.1–10.6 billion people, and by 2100, 12.7–15.6 billion inhabitants (Leridon, 2020). One of the main problems arising from this population increase is how to feed all the inhabitants of the planet, especially under the uncertainties derived from the present and future climate change scenario. It is estimated that the food demand will increase by 60% by 2050, which will lead to the need of a 70% increase in agricultural productivity (Molotoks et al., 2021; Van Dijk et al., 2021).

This necessity to increase agricultural productivity comes up against several factors. The expected effects of the climate change, extreme weather events, droughts, floods, aridity, may limit plant fitness and may increase the incidence of pests and diseases (Hristov et al., 2020). This fact, in addition to the stagnation of agricultural productivity in more than a quarter of our croplands (Ray et al., 2012) and the

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emergence of new pathogens and pets as a result of the globalization (Fones et al., 2020), pose important limitations to crop yields (Poveda, 2021a). Currently, it is estimated that 45% of crops are grown in areas with periods of total absence of precipitation (drought), and that 38% of arable land is affected by salinity, value which increases 1% yearly (2 million ha) due to human action (Yadav et al., 2020). Regarding crop diseases and pests, it is estimated that 20–40% of world agricultural production is threatened by pathogens (10–15% of direct losses), animals, especially insects (18–25%), and weeds (Poveda, 2021b; Mohammad-Razdari et al., 2022).

In order to increase and maintain agricultural productivity in recent decades, the massive use of synthetic chemical fertilizers and pesticides (commonly known as agrochemicals), has mainly been conducted (Tripathi et al., 2020). This massive uncontrolled use is causing serious negative impacts on the environment and biodiversity, soil (harm to the microbial community, accumulation of heavy metals, salinization, etc.), aquifers (eutrophication, toxicity, heavy metals, etc.) and human health (causing diseases, such as cancer) (Hossain et al., 2022). Due to these important problems, different countries are performing important legislative changes to reduce or even prohibit the use of certain agrochemicals. A clear example of this new regulation is the European Green Deal, which includes the “From Farm to Fork” strategy, aiming to reduce the use of chemical pesticides by 50% and of chemical fertilizers by 20% by 2030 (Montanarella and Panagos, 2021). In this sense, there are several strategies which may allow to reduce the use of agricultural agrochemicals without decreasing, or even increasing, crop productivity, such as organic agriculture, use of biofertilizers, integrated pest management, or use of biological control agents (Hossain et al., 2022).

In this scenario, the current and increasingly growing development of a new “livestock” industry, such as insect farms, opens up the possibility of obtaining new products for agricultural use, more sustainable and environmentally friendly. In particular, the industry of mass production of the beetle *Tenebrio molitor* (mealworm), and its great current expansion all over the world, brings the opportunity to exploit a new resource, the frass, the name given to the insect excrement, for agricultural use as both an organic fertilizer and a stimulant of plant tolerance under abiotic stresses and resistance against biotic stresses. The aims of this work are (I) to give an overview of the situation of the mealworm and mealworm by-products industry, with a special focus on frass, and (II) to collect, collate and analyze the main articles published until 2023 on the benefits of mealworm frass for plant production and plant protection. The possibilities of frass to become a real alternative to the use of synthetic agrochemicals, contributing thus to the development of a more sustainable agriculture, and its future perspectives are also evaluated.

2. Insect farming as a generator of new resources

Over the past decade, the prices of soybean meal and fishmeal-primary feeds for livestock— have consistently increased along with the growing demand for animal protein products. Given the challenging scenario for food production, insect farming has emerged as an alternative to uphold food security. Numerous studies indicate that insect meal could be a viable substitute for the meals currently used in animal feeding, offering a strong potential alternative (Sogari et al., 2023). From a scientific perspective, the utilization of insects as both food and feed generates hundreds of publications annually, undergoing continuous review (Sogari et al., 2023; Hawkey et al., 2020).

Edible insects are nothing new. In fact, they currently feed two billion people worldwide (Ordoñez-Araque and Egas-Montenegro, 2021). Contradictorily, in Eastern countries, where entomophagy is a core part of the people diet, insect farming has been almost unknown until recently. Traditionally, in these areas, insects have been obtaining by harvesting from the natural environment (Żuk-Gołaszewska et al., 2022). However, multiple reasons, such as land conversion for agricultural use, desertification and urbanization of natural environments, are causing a decline in the insect captures (Cadinu et al., 2020). Hence, insect farming is advocated as a sustainable alternative which offers several advantages, such as the ability to control environmental conditions, improving production (Madau et al., 2020).

The greatest increase in the development of insect farming industry has been produced in Europe and United States, where there are still challenges to be faced in offering a consolidated product in the feed and food markets (Madau et al., 2020). Some of these challenges include process automation, reduction of the substrate cost and the seeking process of patents (van Huis, 2020). Although there is also another challenge regarding the consumer acceptance, several studies, such as the one conducted by Giotis and Drichoutis (2021), have shown that the perception towards insects as feed is more positive than consuming insect-based foods.

Legal regulations are one of the key requirements to be taken into account in mass-producing insects, in order to ensure their security as feed and food. Due to the aftermath of the Bovine Spongiform Encephalopathy crisis, the use of processed animal protein (PAP) in the feeding of animal farming was banned for almost two decades (European Commission, 2001; European Commission, 2021c). This ban has slowed the expansion of a large-scale insect production for animal feeding. Nevertheless, in recent years there have been two major milestones: in 2017 the European Commission allowed the use of insects for aquaculture, pet and fur animal feeding, and in 2021 for pigs and poultry (European Commission, 2021c).

In addition, other forms of use of insects in feed than the use of PAP were allowed. From Commission Regulation (EU) 2022/1104 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials, live insects and hydrolyzed insect proteins are included as feed sources for pigs, poultry, aquaculture, pet and fur animals (European Commission, 2022b). With regard to the novel food regulation, according to Commission Implementing Regulation (EU) 2021/882, dried larvae of *T. molitor* shall be included in the Union list of authorized novel foods established by Commission Implementing Regulation (EU) 2017/2470 (European Commission, 2021a). Subsequently, as from Implementing Regulation (EU) 2022/169, the placing on the market of frozen, dried and powdered forms of the yellow mealworm as novel food is authorized (European Commission, 2022a). Undoubtedly, all these legislative changes also reinforce the potential of insects as a “new” source of animal feed and as a novel food.

The search for alternative nutrients for feed and food, with insects playing a key role, is linked to several positive impacts, including improvements in sustainability of the production process, the emergence of new economic opportunities, and the potential for establishing new industries in rural areas (de Carvalho et al., 2020). The favorable effects of producing insects for food or feed, including reduced water and land use and notably lower greenhouse gas and ammonia emissions compared to conventional livestock production, have been documented (Hawkey et al., 2020). Additionally, insects present a reduced risk of transmitting zoonotic infections (Lange and Nakamura, 2021). The expected surge in demand for animal protein to sustain a growing population necessitates exploring alternative solutions that avoid further biodiversity loss and the conversion of natural areas into cultivated land. So, insect farming emerges as a viable option in this context (Żuk-Gołaszewska et al., 2022).

Several features render an insect interesting from a production standpoint. On this matter, the optimal species for large-scale production should exhibit swift growth, a diverse diet, minimal chitin content, efficient feed conversion, a brief reproductive cycle, low maintenance demands, and the ability to thrive in crowded environments while displaying high resistance to disease (Żuk-Gołaszewska et al., 2022; Hawkey et al., 2020). Black soldier fly (*Hermetia illucens*), yellow mealworm (*Tenebrio molitor*) and common house fly (*Musca domestica*) are the most promising species for animal feeding replacements according to several studies (Sogari et al., 2019). However, extensive research and commercial attention have been directed toward yellow mealworms, driven by their possession of several desirable traits that make them an ideal candidate for mass production. *T. molitor*, besides being extensively studied among edible insects, stands out for its ease of cultivation and low handling demands. With a high reproductive rate and rapid growth, these characteristics make it particularly favorable for commercialization, as highlighted by Rumbos and Athanassiou (2023). *T. molitor* farming stands out as one of the most promising ventures in the swiftly expanding global market for edible insects. Already being produced on a relatively large scale for use in fish and bird feed, *T. molitor* is also recognized as one of the most consumer-accepted insect species for human consumption. The larvae of *T. molitor* are notably more appealing to European residents compared to options like cockroaches or ants (Gkinali et al., 2022). Due to its importance, the present review has focused on the yellow mealworm (*T. molitor*).

3. Insect frass as agricultural resource

The mass production of the insect rearing industry generates several by-products which should be properly managed for their disposal. The use of these by-products in other industries could valorize them in order to get a higher profit, or at less to reduce the cost of their disposal. According to this, fats (extracted during the defatting process of insect products), chitin, and residual substrates, all of them by-products of the insect rearing industry, can be converted into surfactants and biodiesel, plant protection agents, and fertilizers, respectively (Sogari et al., 2023).

A particularly important by-product, because of the large quantities in which it is generated, is frass. The insect frass, considered as a collection of the insect excrements, is actually a blend of unconsumed substrate, feces, and exuviae. It contains significant quantities of nutrients and beneficial microbes, that makes this product very suitable to be used as fertilizer (Beesigamukama et al., 2023). In a context where legislation is becoming more restrictive on the use of agrochemicals, insect-derived products, such as frass, can be an alternative for the development of sustainable biofertilizers (Barragán-Fonseca et al., 2022). Unlike what happens with another biofertilizers, such as compost, vermicompost, and even bokashi fermentation products, the capacity of insect frass to improve soil health and crop productivity remains not completely understood (Bloukounon-Goubalan et al., 2021). Research on the application of frass fertilizer derived from edible and commercial insects as an organic fertilizer is mostly still scarce, except for *H. illucens* and *T. molitor* (Beesigamukama et al., 2022). The research by Beesigamukama et al. (2022) compares the frass from nine insect species (*H. illucens*, *Gryllus bimaculatus*, *Scapsipedus icipe*, *Bombyx mori*, *Gonimbrasia krucki*, *T. molitor*, *Schistocerca gregaria*, *Pachnoda sinuata* and *Oryctes rhinoceros*), reporting that not only the frass of *H. illucens* and *T. molitor* have adequate contents of macronutrients and micronutrients. The study revealed that among the analyzed species, *T. molitor* frass exhibits the highest Mg and P content. Additionally, it has an ammonia content 3000 times lower than *H. illucens* frass and ranks second only to soldier fly frass in N content (Beesigamukama et al., 2022).

Although the nutritional composition of frass mostly depends on the insects diet, in general, it can be characterized by a high organic matter and a high abundance of nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), along with a narrow carbon (C) to N ratio (Watson et al., 2021a). The nitrogen supply by frass is one of the most noteworthy aspects of its potential use as a fertilizer (Poveda, 2021c). This nutrient richness makes insect frass to be a suitable base ingredient for growth medium in the cultivation of microalgae (Steinrücken et al., 2023). Besides the supply of macronutrients to plants, frass has also been found to enhance of the microbial biomass to soil and to add biomolecules and microbes that promote plant growth (Watson et al., 2022), by either direct growth stimulation or helping plants to cope with biotic and abiotic stresses (Poveda, 2021c). A clear example of that is the presence of chitin in frass, substance that, in the case of *H. illucens,* has been shown to activate the plant defenses against *Fusarium* wilt disease (Quilliam et al., 2020). Regarding the microbial diversity provided by frass, it has been found to be considerably variable as influenced by several factors such as environmental habitat, developmental stage and host phylogeny (Poveda, 2021c). Among the microorganisms occurring in the insect exuviae, a high diversity of chitinolytic bacteria has been identified. This fact might be very interesting as several bacteria of this type, such as *Bacillus thuringiensis, B. cereus* or *Lysinibacillus sphaericus*, have already been marketed for biological pest control due to their antagonistic capacity towards different plant pathogens (Barragán-Fonseca et al., 2022). However, most of these microbes may disappear after the heat treatment of at least 70 °C for 1 h, that is currently compulsory for frass to be placed in the European markets, once the Regulation 2021/1925 of the European Commission has included this insect product into Annex I of Regulation (EU) No 142/2011 (European Commission, 2021b).

4. Yellow mealworm (*Tenebrio molitor* L.): biology, industry and resources

4.1. The biology and lifecycle of mealworms

The *T. molitor* production industry is one of the most promising and fastest growing species in the edible insect rearing industry. Moreover, it is the first insect species approved by the EU to be used as human food (Toviho and Bársony, 2022). *T. molitor* (known as “yellow mealworm”) belongs to the family Tenebrionidae, the fifth largest family in the order Coleoptera, composed by around 20,000 species (Eriksson et al., 2020). This insect was historically considered a pest affecting stored grain, before being considered a very interesting species for feed and food production (Moruzzo et al., 2021).

This insect is holometabolic, developing through four different stages: eggs, larvae, pupae and adults (Gkinali et al., 2022) (Fig. 1). The time to complete the whole cycle is highly variable, from 60 days to even 1–2 years, depending mainly on temperature, humidity, diet and population size. Eggs, measuring 1.7–1.8 mm in length and 0.6–0.7 mm in width, exhibit a glossy, grain-shaped appearance. They are coated with a sticky liquid and can be found either laid individually or in clusters. The time required for hatching of the larvae is highly dependent on temperature, being between 7 and 15 days at 25 °C (Gkinali et al., 2022; Parsa et al., 2023).

The larvae are initially pale whitish in colour, turning yellowish as time goes by. The size of larvae is usually about 1.2 mm–3.5 cm or more (Selaledi et al., 2020; Hong et al., 2020). After the larval period, the pupal stage begins, which lasts approximately 10–20 days at a temperature of 27 °C (Parsa et al., 2023). The pupae, light brown in colour, lack both a mouth and anus, condition that persists until they undergo the transformation into adults. In the adult stage, a brown beetle emerges from the pupa (between 1.5 and 1.8 cm long), which will darken along time. They can live in this stage for 16–173 days (Selaledi et al., 2020).

4.2. Resources and nutritional profile of *T. molitor*

Mealworm larvae can be utilized both live and in the form of meals and oils, serving as a partial substitute for certain conventional ingredients, such as soy, fish, maize, and wheat meal/oil. Many authors have documented the feasibility of substituting traditional fishmeal with mealworm meal in aquaculture production, particularly at inclusion rates of up to 25% (Shafique et al., 2021). It is also a good substitute for soybean meal and has been shown to be suitable for replacing small amounts in poultry and swine diets (Selaledi et al., 2020; Hong et al., 2020). Nevertheless, the utilization of this insect is currently constrained in its ability to control toxins and heavy metals derived from *T. molitor* itself (Hong et al., 2020). Regarding its potential in human food, numerous research studies have evaluated the use of mealworms as an ingredient in well-known food products, including bakery items, such as bread, biscuits, snacks and protein bars, with good results (Moruzzo

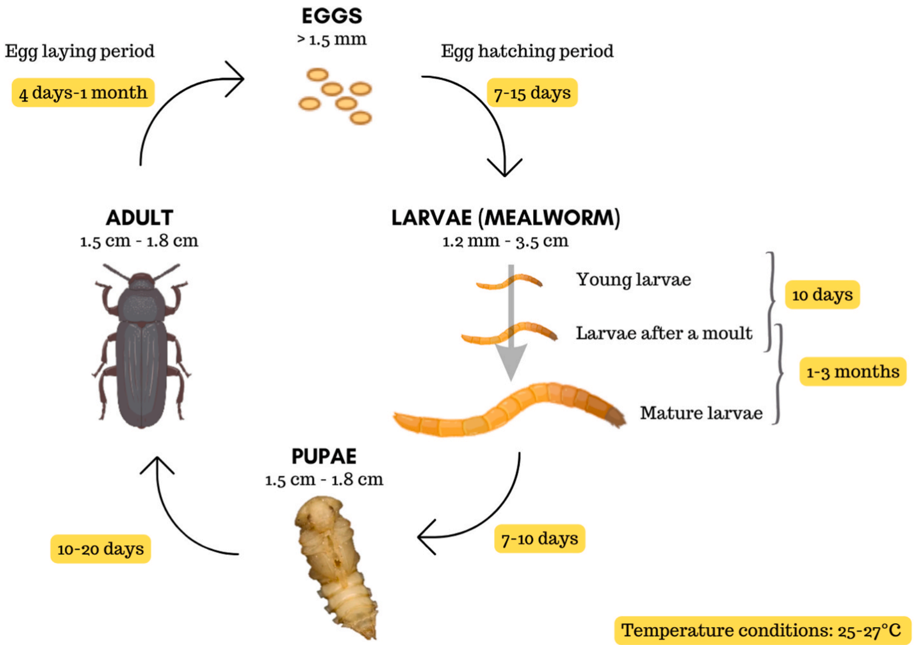

Fig. 1. Life cycle of *T. molitor*.

et al., 2021). Also, the oils extracted from larvae can be used as an ingredient in nutrition, given its similar characteristics to vegetable oil (Errico et al., 2022).

One of the most interesting characteristics of this insect from a feeding point of view, is its proper nutritional profile. On average, 50% of its dry matter corresponds to crude protein and 30% to fat, although variable depending on the diet. In general, mealworm larvae have a well-balanced profile of essential and non-essential amino acids. They are also a suitable source of saturated (such as myristic, palmitic and stearic acids) and polyunsaturated (oleic, linoleic and linolenic acids) fatty acids. It also has a good composition of minerals and vitamins, such as phosphorus, potassium, magnesium, zinc, iron and copper, as well as vitamins B_5, B_3, B_2, B_{12} and E (Moruzzo et al., 2021). The mealworm oil contains tocopherol and polyphenols, both with a strong antioxidant activity (Errico et al., 2022). According to the study carried out by Toviho and Bársony (2022) the age of the larvae might not affect the nutritional value of the resulting feeding product.

As highlighted in a study by Radwan et al. (2023), the potential for utilizing mealworm frass in animal feed has been demonstrated too. Incorporating frass into the diet of rabbits had positive effects, with no adverse outcomes, enhancing both growth performance and parameters related to meat quality (Radwan et al., 2023). The presence of linoleic and linolenic acids in refined oil obtained from *T. molitor* larvae reduces transepidermal water loss and regenerates the lipid barrier of the epidermis, having a suitable profile for use in cosmetics (Verheyen et al., 2023).

Chitin, also known as poly-N-acetylglucosamine, is an insoluble aminopolysaccharide found in the exoskeleton of insects (Errico et al., 2022) and is the most abundant biopolymer on earth after cellulose (Moruzzo et al., 2021). The extraction of chitin and chitosan from insects has proven to be more advantageous over current commercial sources, with advantages in terms of extraction methods, chemical consumption, time efficiency and yield (Moruzzo et al., 2021). In fact, two studies indicated the strength of *T. molitor* as a source of chitin and chitosan. One of them showed that the thinner texture of mealworm chitin had better anti-inflammatory effects (Son et al., 2021). Another study reported by Shin et al. (2019), showed that the composition of chitosan from *T. molitor* had very similar characteristics to commercial chitosan, showing properties of inhibiting the growth of microorganisms, such as *Bacillus cereus, Listeria monocytogenes*, and *Escherischia coli*. *T. molitor* exhibits diverse uses, also serving as biological factories for human probiotics, as illustrated in the experiment conducted by Milanović et al. (2021). This study found an increase in size of *Bacillus clausii* colonies within the intestines of the insect.

The potential of *T. molitor* is very diverse, also as a waste manager. The application of entomoremediation for plastic removal from the environment may be a valuable tool in the future. An illustrative study by Bulak et al. (2021) showcased the larvae and even adults' capability to degrade plastics. Another recent investigation indicated that the larvae of yellow mealworm can sustain themselves by consuming a variety of non-hydrolysable plastics as their exclusive diet (Yang et al., 2023). Mealworms emerge as potential candidates for the upcycling of plastic waste, contributing to the production of protein sources (Yang et al., 2023).

In addition to entomoremediation, the use of agro-food waste can be a solution to make the process more efficient, environmentally more interesting and with a circular economy perspective. As highlighted by multiple authors, *T. molitor* is capable of thriving on low-value organic waste substrates and converting them into high-value products for the market (Moruzzo et al., 2021; Errico et al., 2022). However, the use of agri-food waste as feed for mealworms has certain limitations, such as being free of contamination, including pathogens and heavy metals, and should not compete with feed for other animals.

It should also be considered the seasonality of agricultural residues, as this factor makes maintaining a consistent supply challenging (Moruzzo et al., 2021). Additionally, it is anticipated that their prices will rise as agricultural practices progress towards greater circularity, leading to an increased demand for these by-products (Niyonsaba et al., 2023).

4.3. Industrial processes

Insect rearing activities cover the following production steps: providing substrates to insects, facilitating its growth phase, harvesting the insects, and implementing pre-treatment steps. Regarding the feeding substrates, they affect feed conversion efficiency, as well as mealworm development and growth. It has been shown that the largest larvae sizes are achieved when they are feeding with wheat bran (Selaledi et al., 2020), although the use of different kinds of flour (wheat, oats, maize) has also provided good results (Kooh, et al., 2020). Despite its resistance to dryness, it is advisable to supplement the diet with fruits and vegetables to provide a source of water (Kooh et al., 2020). The mealworm growth phase must be carried out in a close environment, controlling temperature and relative humidity conditions. Proper cleaning and sanitary measures must be implemented to prevent contamination or the spread of diseases within the rearing flocks (Hong et al., 2020). For harvesting, after a 24-h starvation period to clean the gastrointestinal tract, the fully grown larvae are sieved to separate frass and substrate (Kooh et al., 2020). Around 60 days after hatching is suggested as an optimal harvesting period for mealworm larvae intended for use in fish feed (Rodjaroen et al., 2020).

The International Platform of Insects for Food and Feed (IPIFF) has stated that insects should be subjected to different processing methods during slaughtering and after for their utilization in animal feed (Hong et al., 2020). They are frequently euthanized using methods, such as hot water, boiling vapor or freezing. It is advisable to blanch the insect before the slaughtering process, as it helps to slow down bacterial growth during storage. After this phase, freezing, refrigeration and drying can be carried out, being this latter considered very important due to the high moisture content of the larvae (68%). The main drying methods are oven drying, vacuum, freeze-drying or microwave drying (Hong et al., 2020). Most insect applications in animal feed need a grinding/milling procedure to convert the insects into fine particles, creating a homogeneous powder. The process called fractionation involves the application of physical, chemical, and biochemical methods to extract proteins, fat/oil, chitin, and derivatives (such as chitosan) as the final output of the process. Different methods can be used to obtain the fat, such as pressing, heat treatment or the use of organic solvents. The extraction of chitin involves chemical and/or enzymatic processing (IPIFF, 2022).

5. Analysis conducted

A literature review was conducted along with a quantitative analysis of publications based on the year, journal and countries. The compilation of all publications was carried out using the following keywords "(mealworm OR *Tenebrio molitor*) AND (frass OR excreta OR feces) AND (agriculture OR crops OR plant OR fertilizer)". The bibliographic databases Web of Science™ (Web of Science Core Collection - WoS) (http://www.webofscience.com) and the Elsevier® Scopus library services metabase (http://www.scopus.com) were utilized. This choice was made due to the advantages of scientific rigor compared to other free and more open databases, such as Google Scholar (Martín-Martín et al., 2021).

In WoS, after searching the keywords in "All Fields", without time restrictions, a total of 69 results were retrieved (search conducted on December 24, 2023). Of these 69 articles, 49 were not directly related to the subject, four other articles were reviews (Ravi et al., 2020; Poveda, 2021c; Chavez and Uchanski, 2021; Moruzzo et al., 2021), and another one was an editorial (Watson et al., 2022). Therefore, 15 articles were included in the review. On the other hand, after searching for keywords in "Title, Abstract and Keywords", without time restrictions, a total of 31 results were retrieved in Scopus (search conducted on December 24, 2023). Out of these, 14 were not directly related to the subject of this work, one other article was a review (Moruzzo et al., 2021), and another one was an editorial (Watson et al., 2022). Therefore, 15 articles were included in the review. It is important to note the overlapping results between the two databases. Thirteen out of the 15 articles found in WoS and Scopus were coincident. Therefore, each database provided two additional articles, accounting a total of 17 articles (13 from both databases, plus 2 only present in WoS, plus another 2 only present in Scopus) included in the present review on the yellow mealworm frass uses in agriculture.

Although the first article about the effect of mealworm frass on plants was in 2013 (Li et al., 2013), afterward there was a gap without publications until 2019 (Poveda et al., 2019). From 2019 to 2023, several publications were made (totaling 16 articles), with the years with the highest number of publications being 2021 (5 articles) and 2023 (7 articles). The field of study of the use of insect frass in agriculture is something very novel and intensively addressed in recent years (Poveda, 2021c), as is the case with the study of mealworm frass as an agricultural resource, with an increase in publications from 2019 to 2023. Regarding the country origin of the publications, France and Spain stand out as the countries with more articles (3 articles), followed by China and Germany with 2 articles. With one article published, a total of 8 countries are found from Europe (Greece, Norway, Poland and The Netherlands), America (Argentina and Canada), Africa (Zimbabwe) and Asia (Singapure).

6. Mealworm frass as agricultural resource

The insect farming industry of yellow mealworm is especially aimed at the production of raw material for feed and food. However, there are other by-products of great interest derived from the massive rearing of these insects that can have profitable and sustainable applications. This is the case of mealworm frass, a very important by-product of this insect farming, since to produce 100 g of mealworm-biomass, 200–300 g of frass are generated (He et al., 2021).

Several works have studied the benefits that mealworm frass can have in agriculture, with a remarkable interest in the last 5 years (2019–2023). Table 1 compiles and classifies all these works, indicating the plant/crop used, the effects caused, and the mechanisms of action involved. There are two major types of effects and mechanisms, as summarized in the infography of Fig. 2: promotion of plant growth and productivity and a phytofortifier effect against abiotic and biotic stresses. Both types are presented and discussed in detail in the following two subsections.

6.1. As an organic fertilizer: promotion of plant growth and productivity

When applied to soil or growing substrate, mealworm frass has been described as an efficient nutrient-rich organic fertilizer (Fuertes-Mendizábal et al., 2023). Application of mealworm frass to the soil could provide with the necessary amounts of P and K, in a readily available form, the crop development requires. It is also an important source of N, which seems to be slowly mobilizable from the frass to the soil, e.g., with an NPK of 5-2-1.7 (Houben et al., 2021). Nevertheless, the speed in the mobilization should be analyzed in terms of the crop capacity to uptake the released mineral. For instance, in an *in planta* study conducted on ryegrass, it was found that N release from mealworm frass was too fast compared to the plant's ability to assimilate that N (frass NPK: 4-1.5-3). Therefore, the authors proposed the addition of nitrification inhibitors along with the frass to increase the plant N uptake and minimize the N losses (Watson et al., 2021b). In a study conducted on horticultural peat as a substrate, the use of frass (dosage: 5–10 g/dm^3) significantly increased the N content of the growing substrate, rather than other nutrients, such as P, K, Mg and Na, and caused a decrease of the Ca content, after 2 weeks of the application (Nogalska et al., 2022). Another study, which analyzed the results of the application after a longer period (4 weeks), also reported an increase in soil mineral N content released from mealworm frass (dosage: 5 %, v/v; frass NPK: 2.3-2.6-10) (Watson et al., 2021a). Similarly, composting may also increase the N release from frass, such as it has been indicated by He et al. (2021) after a 32-day composting of *T. molitor* frass. Therefore, the higher or lower release of nutrients, especially N, to the soil by mealworm frass seems to be determined by the time it remains in the soil. Nevertheless, the N dynamic from mealworm frass to soil and plant might be complex, being necessary a deeper study, including different types of soil and crops, to clarify it.

The possibility of using mealworm frass as an effective organic fertilizer in agriculture is a quite novel idea developed in recent years. The first *in planta* study was developed in chard with a frass characterized by a NPK 3-1.5-2 profile, which also contained micronutrients essential for

Table 1
Studies of the use of frass from *T. molitor* as agricultural resource.

CROP/ PLANT	EXPERIMENT CONDITIONS	FRASS DOSAGE AND FORMAT USED	EFFECTS	MECHANISMS OF ACTION	REFERENCES
–	In soil	Frass (10 t/ha)	Increased soil nutrient content	Nutrient supply Improvement in microbial activity	Houben et al. (2021)
–	In soil	Frass (5 %, v/v)	Increased soil nutrient content Reduction of heavy metal content of the soil	Nutrient supply Increase microbial abundance Biosorption of heavy metals	Watson et al. (2021a)
–	In horticultural peat	Frass (5–10 g/dm^3)	Increased soil nutrient content	Nutrient supply Increase microbial abundance	Nogalska et al. (2022)
–	*In vitro*	Frass bio-oil (200–400 ppm)	Insecticidal effect (repellent and larvicidal)	Presence of insecticidal metabolites	Urrutia et al. (2023)
Arabidopsis	*In planta* (in pots, growth chamber)	Frass (2 %, v/v)	Activation of systemic defenses against the necrotrophic pathogen *Botrytis cinerea.*	Presence of chitin	Blakstad et al. (2023)
Arugula	In field	Frass (0.5 %, v/v)	Increase edible biomass (leaf)	Nutrient supply	Hénault-Ethier et al. (2023)
Barley	*In planta* (in pots, greenhouse)	Frass (10 Mg/ha)	Plant growth promotion	Nutrient supply	Dulaurent et al. (2020)
	In planta (in pots, greenhouse)	Frass (10 Mg/ha)	Plant growth promotion	Nutrient supply Improvement in microbial activity	Houben et al. (2020)
Bean	*In planta* (in pots, greenhouse)	Frass (2 %, v/v)	Increase abiotic stress tolerance (salinity, drought and flooding)	Presence of chitin Presence of tolerance-inducing bacteria and fungi (only under flooding)	Poveda et al. (2019)
Beetroot	In field	Frass (0.5 %, v/v)	Increase edible biomass (root)	Nutrient supply	Hénault-Ethier et al. (2023)
Brussels sprouts	*In planta* (in pots, greenhouse)	Frass (5 g/kg)	Plant growth promotion	Presence of plant growth-promoting bacteria	Van de Zande et al. (2023)
Chard	*In planta* (in pots, greenhouse)	Frass (2 %, v/v)	Plant growth promotion	Nutrient supply Presence of plant growth-promoting bacteria and fungi	Poveda et al. (2019)
Cherry tomato	In field	Frass (0.5 %, v/v)	Increase edible biomass (fruit)	Nutrient supply	Hénault-Ethier et al. (2023)
Cucumber	In field	Frass (0.5 %, v/v)	Increase edible biomass (fruit)	Nutrient supply	Hénault-Ethier et al. (2023)
Dragon fruit cacti	*In vitro*	Frass (100% of the culture substrate)	Reduction of aerial growth Increased rooting	Not identified	Gan et al. (2021)
Dwarf sunflower	In field	Frass (0.5 %, v/v)	Increase flowers number	Nutrient supply	Hénault-Ethier et al. (2023)
Kale	In field	Frass (0.5 %, v/v)	Increase edible biomass (leaf)	Nutrient supply	Hénault-Ethier et al. (2023)
Lettuce	*In planta* (in pots, greenhouse)	Frass (1 %, v/v)	Plant growth promotion	Nutrient supply	Fuertes-Mendizábal et al. (2023)
Nasturtium	In field	Frass (0.5 %, v/v)	Increase flowers number Increase edible biomass (leaf)	Nutrient supply	Hénault-Ethier et al. (2023)
Ryegrass	*In vitro*	Composted frass (doses not indicated)	Increase germination index	Nitrogen supply	He et al. (2021)
	In planta (in pots, greenhouse)	Frass (1.5 %, w/w)	Soil nitrogen increase Increase in plant nitrogen Increase in soil-microbial biomass	Nitrogen supply	Watson et al. (2021b)
Spinach	*In planta* (in pots)	Frass (1 %, w/v)	Plant growth promotion	Nutrient supply	Antoniadis et al. (2023)
Sunflower	*In planta* (in pots, greenhouse)	Frass (5 %, v/v)	Plant growth promotion	Nutrient supply	Blakstad et al. (2023)
Sweet corn	In field	Frass (0.5 %, v/v)	Increase edible biomass (fruit)	Nutrient supply	Hénault-Ethier et al. (2023)
Tomato	*In planta* (in pots, greenhouse)	Frass (2 %, v/v)	Plant growth promotion	Nutrient supply	Blakstad et al. (2023)
Wheat	*In vitro*	Aqueous extracts (0.4 mL/seed)	Not improve seeds germination Reduction in plant growth	Presence of fatty acids with phytotoxic capacity	Li et al. (2013)
	In planta (in pots, in room)	Frass (2 %, w/v)	Plant growth promotion Increase yield	Nutrient supply	Nyanzira et al. (2023)
Zinnia	In field	Frass (0.5 %, v/v)	Increase flowers number	Nutrient supply	Hénault-Ethier et al. (2023)

plant growth and development (S, Ca, Mg, Mn, Fe and Mo). Possibly due to the nutrient supply by mealworm frass application (dosage: 2 %, v/v), chard plants increased their chlorophyll content, fresh weight, aerial part length and collar thickness (Poveda et al., 2019). This plant growth promotion derived from a possible nutrient supply by mealworm frass application has been described in many other plants and crops. In cereals, the application of mealworm frass as fertilizer increased the biomass developed by barley (dosage: 10 Mg/ha) (Houben et al., 2020) and the grain yield produced by wheat (dosage: 2 %, w/v) (Nyanzira et al., 2023). This fertilizing effect of mealworm frass might be significantly enhanced if earthworms are applied simultaneously, as the releases of nutrients from frass might be accelerated by the worms (Dulaurent et al., 2020). In addition, it is interesting to note that the type of feeding of mealworms conditions the characteristics of the frass obtained, both in terms of nutrients and microbial diversity (Poveda et al., 2019).

The positive results produced by mealworm frass as organic fertilizer in the enhancement of crop production are clearly influenced by its application rate. In lettuce, it was determined that frass doses of 1% v/v provided all the necessary macronutrients for the crop, increasing

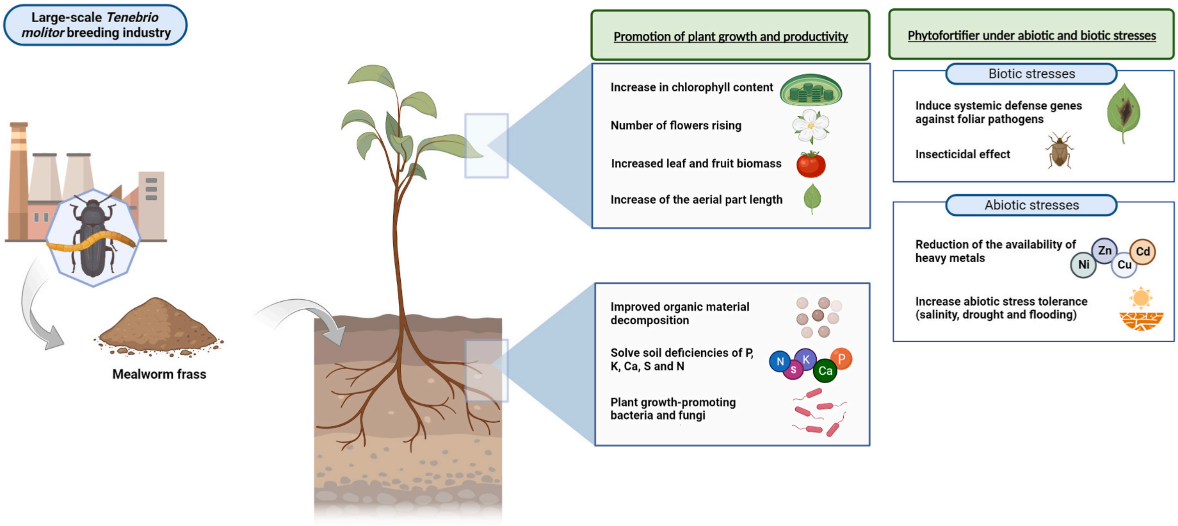

Fig. 2. Infographic summarizing all the effects and mechanisms of action of frass from *T. molitor* in its agricultural use.

lettuce aerial biomass. However, increasing the doses by 2.5% v/v reverted the positive effects on plants into negative, causing a decrease in their growth, especially in the root system (Fuertes-Mendizábal et al., 2023). This negative effect related to the dose could be a consequence of an increase in the presence of pathogenic fungi, as indicated by the authors (Fuertes-Mendizábal et al., 2023), which could be favored by the contribution of nutrients and chitin provided by the mealworm frass. Similar results were obtained by Antoniadis et al. (2023) on spinach at doses of 1% w/v. Nevertheless, the optimal doses rate seems to be related to the crop type, as in the same study by Antoniadis et al. (2023) was found that, for leaf crops, the contribution of N from the frass might be more relevant than that of P and K. In another quite different type of crop, like sunflower, the application of mealworm frass was able to remedy different nutritional deficiencies in soil, especially those of P, K, Ca, and S, although it was not able to fully compensate the absence of N in soil (Blakstad et al., 2023).

All the studies indicated so far showing the efficiency of mealworm frass as organic fertilizer has been developed under controlled greenhouse conditions. Although such studies are suitable to give a first approximation of the fertilizing capacity of mealworm frass, field studies might also be required to know the actual scope of its use at a large scale. In this sense, Hénault-Ethier et al. (2023) have developed an extensive study where mealworm frass is applied to a wide variety of crops in the field. As main results, they obtained that, in ornamental plants, such as dwarf sunflower, nasturtium and zinnia, the application of a 0.5% v/v dose of frass to the soil significantly increased the number of flowers formed on these plants. In vegetable crops, the frass application significantly increased the biomass of the edible part of beetroot (increased root biomass), arugula, kale and nasturtium (increased leaf biomass), and cherry tomato, cucumber and sweet corn (increased fruit biomass). Therefore, this in-field study (Hénault-Ethier et al., 2023) may support the suitability of mealworm frass to be used as organic fertilizer in multiple crops, already observed under greenhouse conditions.

Therefore, mealworm frass can be an effective way to provide nutrients to crops. However, the nutrient profile is very different in each of the studies, probably due to the feed provided to the insects, although this is an aspect that requires significant future research. To consider as a whole the role that the feed source has on the nutritional profile of mealworm frass, Table 2 lists the information from the studies in this regard.

Besides the supply of nutrients by frass, the presence of plant growth-promoting bacteria in its composition has also been described (Van de Zande et al., 2023). Those bacteria, present in the rhizosphere of Brussels sprouts growing in a bacteria-free substrate after the mealworm frass application, were regarded to be involved in the growth promotion observed in the plants (Van de Zande et al., 2023). Similarly, other works have identified how nutrient delivery from mealworm frass to the soil is accelerated by an increase in soil microbial abundance and activity after insect frass application, both in bacteria and fungi capable of decomposing organic matter and mineralizing N (Houben et al., 2020, 2021; Watson et al., 2021a; Nogalska et al., 2022). Despite the role that the microorganisms present in mealworm frass can play a role in the release of nutrients and/or as direct promoters of plant growth, there are cases in which it has been demonstrated that their presence is not essential for the results of the observed increases in plant biomass after its application. In the study developed by Poveda et al. (2019), it was proven how the promotion of chard plant growth by mealworm frass was independent of the presence of plant growth promoting

Table 2
Nutritional profile of frass and source feed provided to mealworm frass during rearing.

NPK PROFILE	MEALWORM FEED	REFERENCE
3-1-2	Raw material unspecified (composition: 66% carbohydrates, which were mainly celluloses and hemicelluloses, 6% fat and 28% protein)	Poveda et al. (2019)
3-1-2	Raw material unspecified (composition: 77% carbohydrates, of which 12% was starch; 6% fat and 28% protein)	Poveda et al. (2019)
8-1-1	Raw material unspecified (composition: 49% carbohydrates, again mainly celluloses and hemicelluloses, and 12% fat and 39% protein)	Poveda et al. (2019)
5-2-2	Not indicated	Dulaurent et al. (2020)
5-2-2	Wheat bran	Houben et al. (2020)
5-2-2	Not indicated	Houben et al. (2021)
2-3-10	Raw material unspecified (composition: 20% protein, 4% fat, 1.01% Ca, 0.76% P and 0.23% Mg), supplemented with carrots	Watson et al. (2021a), 2021b
3-4-9	Wheat flakes, wheat bran, yeast and rice flour, supplemented with carrots	Nogalska et al. (2022)
3-2-3	Wheat bran, supplemented with different food wastes (unspecified)	Blakstad et al. (2023)
4-6-3	Wheat flour, supplemented with vegetables (unspecified)	Fuertes-Mendizábal et al. (2023)
3-3-2	Wheat bran, supplemented with fruit and vegetable pulp juice factory	Hénault-Ethier et al. (2023)
3-2-3	Wheat bran, supplemented with carrots	Nyanzira et al. (2023)

microorganisms, applying autoclaved frass (Poveda et al., 2019). Similar results were obtained in tomato plants (Blakstad et al., 2023). It is important to note that all the studies refer to non-heat-treated frass, therefore, maintaining its microbiota.

Despite the potential use of frass as organic fertilizer, not all the studies performed have evidenced positive results. In fact, in one of the first works on frass (Li et al., 2013), aimed to develop circular food generation processes in a closed system, such as a space station, the application of aqueous extracts from mealworm frass on wheat caused a strong inhibitory effect on germination and plant growth, possibly due to the presence of certain fatty acids (Li et al., 2013), whose profile is closely related to the diet received by the mealworms (Mattioli et al., 2021). This suggests that the frass of *T. molitor* may need a previous treatment before its addition to the cultivation substrate to limit its phytotoxicity (Li et al., 2013). In this regard, the application of frass after composting for 32 days increased the germination of ryegrass seeds *in vitro* (He et al., 2021). Therefore, composting could be a suitable treatment to limit or eliminate any potential negative effect of the frass application, although further research should be performed to support this consideration. In another study carried out to determine the efficiency of *T. molitor* as a degrader agent of plastic materials (Przemieniecki et al., 2020; Zhong et al., 2022), the frass produced by larvae fed with polystyrene as carbon source, had negative effects on the growth of the aerial part of the dragon fruit cacti when used as fertilizer. However, it also enhanced its root development, indicating the possibility of using this plastic-derived frass as a possible fertilizer, but requiring further research (Gan et al., 2021).

6.2. *As a phytofortifier under abiotic and biotic stresses*

Besides the potential use of mealworm frass as biofertilizer, some studies have recently reported a phytofortifying effect of this by-product on crops subjected to abiotic and biotic stresses after its application. Regarding abiotic stresses, the contamination of agricultural soils with heavy metals represents one of the most damaging forms of soil degradation, having a very negative impact on crops, not only because of direct phytotoxicity but also because of the damage it causes on the rest of the organisms in the agrosystem (Vácha, 2021). In this sense, it has been described how mealworm frass can be used in metal-contaminated soils to improve crop establishment, since it presents metal sorption and complexation, significantly reducing the metal bioavailability in the soil, specifically Zn, Cu, Cd and Ni (Watson et al., 2021a).

The ability of mealworm frass to induce plant tolerance under abiotic stresses, such as drought, flooding and salinity, has also been studied. Firstly, it was found that the application of this insect frass on bean plants increased their plant biomass under salinity, drought and flooding (Poveda et al., 2019). Subsequently, by using autoclaved mealworm frass, it was determined that only under flooding stress the bacterial and fungal microbiota present in the frass played a key role in the induction of plant tolerance. However, under salinity and drought the absence of microorganisms did not modify the plant tolerance-inducing capacity of mealworm frass (Poveda et al., 2019). In the latter case, this tolerance-inducing effect could be related to the presence of chitin in insect frass, a polymer widely described to confer tolerance to plants toward salinity and drought stress (Malerba and Cerana, 2020; Pongprayoon et al., 2022).

Likewise, chitin has been also described as an effective inducer of plant defensive responses against biotic stresses (Malerba and Cerana, 2020; Pongprayoon et al., 2022). In this regard, aqueous extracts from mealworm frass were found not to induce a local defensive response in arabidopsis roots (specifically, callose deposition). However, a direct contact of arabidopsis roots with soil-applied insect frass was able to induce systemic defense genes against a foliar attack by the necrotrophic pathogen *Botrytis cinerea*, again probably due to the plant-inducing effect to the presence of chitin in the frass (Blakstad et al., 2023).

Finally, although the potential use of mealworm frass as an insecticide of crop pests has barely been studied so far, it has been known for decades that this product contains volatile fatty acids that act as repellents among the larvae of *T. molitor*, in order to prevent the massive agglomeration of individuals. Specifically, butyric acid, propionic acid and valeric acid were described as the components involved in this mechanism (Weaver et al., 1990). Being able to avoid mass agglomeration around a resource is a fundamental aspect of survival in insects, such as mealworms, otherwise they could die from suffocation and/or starvation. This evolutionary mechanism of yellow mealworm could be used to combat insect pests in agriculture. In this sense, a bio-oil was obtained by pyrolysis of mealworm frass, which presented mostly the compounds acetic acid and 1,6-anhydro-β-d-glucopyranose. This bio-oil was applied on larvae of the lepidopteran pest *Plodia interpunctella* and the coleopteran pest *Tribolium castaneum*, reporting a significant lethal effect only for the lepidopteran. However, in the application of this bio-oil as a repellent, it worked effectively against both the lepidopteran and the pest beetle (Urrutia et al., 2023).

In view of the work carried out so far, it is clear that mealworm frass has great potential for use as an agricultural resource. However, it is necessary to develop regulations that allow its real use within the production system of each country. In this sense, on November 2021, the European Union approved Regulation (EU) 2021/1925 specified the characteristics that mealworm frass must have in order to be placed on the market and used in agrosystems, which has been a very important step in the real use of insect frass as an agricultural resource (European Commission, 2021b).

7. Conclusions and future prospects

The recent modification of the insect mass rearing regulatory framework, which authorizes insect-produced feed for pigs and poultry, will boost the creation of new insect industries, resulting in huge quantities of frass that will have to be revalued. At the same time, regulations are increasingly restrictive on the use of synthetic chemical fertilizers and pesticides in agriculture.

As stated in this review, frass of *T. molitor* has a number of characteristics that make it very suitable for use in organic farming. Its properties as (I) phytofortifier against biotic and abiotic stresses as well as (II) organic fertilizer, have been recently demonstrated. Nevertheless, additional research is required in the realm of agricultural systems to ascertain whether insect frass can serve as a comprehensive replacement for organic fertilizer. One of the possible handicaps is the amount of frass that needs to be produced for this substitution to take place. For the time being, its use in intensive horticultural crops or even high-value woody crops could be considered, but its use in extensive crops is far from being possible. Also, it is essential to conduct new studies on the impact of this product across various types of crops. This includes establishing standardized usage doses and determining the optimal application methods in the field.

A relevant and little addressed aspect so far is the importance that mealworms' feeding has on the agricultural characteristics of their frass. It has been determined that feeding conditions both the nutritional content and the microbiota present. More research is needed on this aspect in order to develop the best feeding protocols based on obtaining the frass with the best characteristics. Other specific future lines of research to be addressed in the future could be with the knowledge of the mechanisms involved in the uptake of frass nutrients by plants or how different frass affect different pathogens and/or pests.

So far, there are many studies that analyze the effect of different food resources on the growth and development of mealworms. However, the effect of mealworm food on the qualities of their frass for agricultural use has been little analyzed. This is an aspect of utmost relevance in the production of a frass with an efficient nutritional and microbial content for agricultural use.

On the other hand, EU 2021/1925 states that, in order to be marketed for agricultural use, frass from mealworm must be treated at least

70 °C for 1 h (European Commission, 2021b). This disinfection process will eliminate most or all of the beneficial microbiota to plants described in the frass. Therefore, the results obtained so far with frass without heat treatment could be very different from the frass actually marketed. In this sense, studies with treated frass or the request for a modification of the heat treatment, following product safety analyses, are required.

In summary, to shed light on these intricate dynamics, it is imperative to broaden the research efforts into the impacts of insect-derived products on interactions among insects, plants, nutrients and microbes. Enhanced comprehension of pivotal factors that influence the successful utilization of mealworm residual streams can contribute to their adoption as an innovative strategy for establishing resilient crop production systems.

CRediT authorship contribution statement

Irene Zunzunegui: Writing – review & editing, Writing – original draft, Investigation. **Jorge Martín-García:** Writing – review & editing. **Óscar Santamaría:** Writing – review & editing. **Jorge Poveda:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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