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Tenebrio molitor: INNOVATIVE TOOL FOR FOOD LOSS AND WASTE VALORIZATION AND BIOPOLYMERS RECOVERY

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Abstract

Modern society is faced with a series of important challenges for its very survival, related to climate change, resource depletion, population increase, soil scarcity, and environmental pollution. It is now clear that the linear economy model, adopted up to now mainly by the most industrialised countries, is no longer sustainable, and urgent alternative solutions are required. The insects, and in particular Tenebrio molitor (TM), are a valid food alternative, and they can be used to valorise and reduce food loss and waste (FLW) in the Circular Economy perspective, converting FLW into high-value products including food, feed, pharmaceuticals, biomaterials, and lubricants. Furthermore, TM rearing waste provides fertilisers and bioproducts, such as chitin and chitosan, as well as biofuels and biochar. TM and its gut microbiota also represent a valid tool for plastic degradation, even though plastic pollution management using TM is quite controversial. Finally, TM can provide valuable assistance in the biological recovery of new biopolymers, such as polyhydroxyalkanoates (PHA) from plastic-producing microorganisms, (e.g. Cupriavidus necator), used as Single Cell Protein. In a circular system and following a bioeconomy approach, these microorganisms can be fed on FLW, produce PHA, and then be used as feed for mealworms to obtain PHA and, at the same time, protein biomass, as well as rearing waste (frass and exuviae) from which to obtain fertilisers for new crops and chitin/ chitosan for biomaterials.

Key words: agri-food by-products, bioplastics, circular bioeconomy, chitin and chitosan, plastic waste

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1. Introduction

Population growth is expected to reach 9.7 billion by 2050 (Worldometer, 2021). New protein sources must be exploited to cope with this population explosion, thus increasing overall food production by approximately 50-60% compared to world production in 2005/2007 (UN, 2019). Food systems throughout the supply chain are responsible for 34% of global greenhouse gas (GHG) emissions (Crippa et al., 2021). According to IPCC (2021), which examined different emission scenarios from 2015 to 2100, the global surface temperature will continue to increase until at least 2050, leading to global warming. This plight risks being aggravated by the increase in food production. Additional global warming can further exacerbate desertification, land degradation, sea-level rise, extreme weather events and natural disasters, thus exacerbating food insecurity conditions, malnutrition, and the gap between the richest and the poorest (FAO, 2016). In addition, the food system generates around 1.3 billion tons of food loss and waste (FLW) per year along the food chain (corresponding to one-third of world food production). Globally, production, post-harvest, and consumption stages contribute to over 80% of the FLW (Xue et al., 2017).

Generating FLW has associated an annual cost of over 1000 billion dollars, along with environmental impacts, including a carbon footprint of approximately 7% of total GHG of anthropogenic origin; a land-use

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of 1.4 billion hectares; a bluewater footprint of 250 km^3 (Wohner et al., 2019). On the contrary, FLW reduction contributes to food sustainability, the efficiency of the food system, and food security (FAO et al., 2019). Added to these problems is plastic pollution. The use of plastic materials increases dramatically, primarily in the packaging sector. In 2019, plastic production was 370 million tons globally and 58 million tons in Europe (Plastics Europe, 2020). Parallelly, there is a tiny improvement in the management of plastic waste. Only 9% of plastic is recycled worldwide (UNEP, 2018). Plastic waste is now in the most distant and extreme environments and enters the food chain through marine fauna as microplastics (Cverenkárová et al., 2021). There is no doubt that plastic waste represents a great opportunity in terms of reuse and recycling from a circular perspective. Furthermore, plastic is now undeniably a necessary and irreplaceable material. On the one hand, therefore, it is urgent to find sustainable systems to manage and enhance plastic waste and, on the other, to seek solutions to produce recyclable non-fossil plastics with good performance, such as polyhydroxyalkanoates (PHA) (Mazhandu et al., 2020). Concerning this dramatic scenario, which raises serious concerns for the future, it is necessary to explore every remedy available.

The mealworm beetle TM represents an alternative source of high-quality proteins for food and feed. It has been recently authorized as the first insect as Novel Food in Europe (Commission Implementing Regulation 2021/882) and approved for feed in aquaculture, pets, pigs and poultry (European Commission Regulation 2017/893). Compared to meat livestock, TM has a high feed conversion ratio and elevated growth rate. TM production emits fewer GHG and requires less water and land (Oonincx et al., 2010; Oonincx and de Boer, 2012). However, the large-scale production of TM larvae (TML) is not yet fully sustainable and uncompetitive compared to conventional livestock. Moreover, the actual product of interest for both food and feed is TML flour/meal, which needs an additional energy-intensive phase for transforming the larvae into flour (FAO, 2021; Maillard et al., 2018). In this way, the environmental and economic costs of TML meals are not competitive compared to other protein meals like soybean meals or fishmeal (Le Féon et al., 2019).

The use of agri-food waste to feed TML increases its sustainability and, at the same time, help manage a great deal of FLW. TM rearing efficiently converts FLW into a wide range of high-value products, such as food, feed, fertilizers, chitin, chitosan, biomaterials, biofuels (Cadinu et al., 2020, Moruzzo et al., 2021a). TM degrades many substrates (Derler et al., 2021) and even plastics due to its highly differentiated microbiota (Przemieniecki et al., 2020). Furthermore, TM can release PHA by lysis of the cell walls of microorganisms by feeding on them (Murugan et al., 2016).

Figure 1 shows the role of TM in the circular bioeconomy. TM valorises FLW and plastic waste and recovers biopolymers from microorganisms. The exploitation of products deriving from TM rearing waste (chitin, fertilizers etc.) can improve production cost-effectiveness.

In this review, we examine the state-of-the-art relating to the use of TM in a circular bioeconomy perspective to solve the environmental and socio-economic problems described so far. We treat the valorization of FLW using TM and examine the different agro-industrial substrates studied to rear this insect. We critically discuss the ability of TM, along with its gut microbiota, to biodegrade plastic waste, on the one hand, and recover PHA biologically and cost-effectively, on the other. Finally, we explore the great potential of TM rearing waste (frass and exuvia) to have fertilizers and chitin/chitosan, respectively.

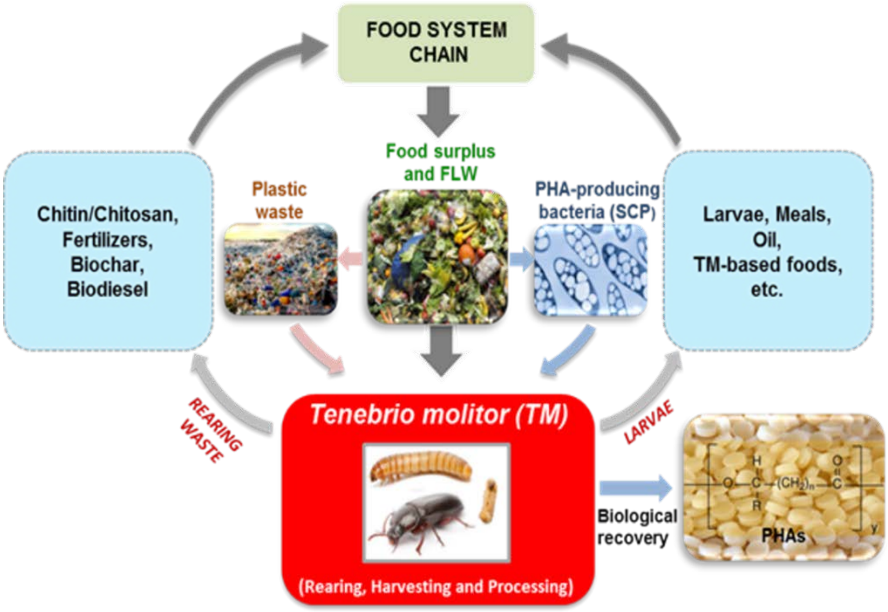


Fig. 1. TM in the Circular Bioeconomy

2. Valorizing and reducing food loss and waste by TM

As demonstrated by several authors, TM can transform low-value substrates into plenty of products with application in many sectors such as food, feed and biomaterials (Errico et al., 2021; Moruzzo et al., 2021a; Ojha et al., 2020). For this capacity, in recent times, TM has become particularly interesting from a circular economy perspective to achieve the zero-waste goal and close the loop of the food value chain (Cadinu et al., 2020; Sangiorgio et al., 2021b).

The use of FLW adds value to poor and environmentally impacting substrates and make TM rearing more sustainable. Indeed, although the TM rearing meets many of the Sustainable Development Goals, it is not yet entirely advantageous either in environmental or economic terms, especially on a large scale (Moruzzo et al., 2021b). Crop residues and food processing by-products are valuable and cheap feed resources (Ravi et al., 2020; Rovai et al., 2021).

The choice and management of the substrate are essential, especially if you want to use TM as food or feed. In this case, Regulation (EU) no. 68/2013 on the catalogue of feed materials requires that insects cannot feed on food contaminated by pathogens, as well as on animal by-products or catering waste. In addition, the use of food by-products must consider the potential contamination of waste with heavy metals, mycotoxins, pesticides or other hazardous materials (FAO, 2021). Moreover, the seasonality, transport, and storage of the substrate chosen for TM rearing are crucial aspects to consider (Sangiorgio et al., 2020). Finally, the choice of feed substrates should not cause competition with other animal productions from a food system sustainability perspective (Pinotti and Ottoboni, 2021). However, since TM rearing is conducted in controlled indoor conditions - requiring no specific geographic or natural environmental conditions - it can be placed close to substrate suppliers, thus reducing handling and costs to a minimum (Mancini et al., 2019). The research evaluates improvements in sustainability and cost-effectiveness of TM biomass production by new feed resources exploration. (Rumbos et al., 2020; Van Peer et al., 2021). Growth parameters, feed efficiency indicators and the nutritional value of TML are some of the parameters used to study the ability of TML to eat different substrates. As extensively reported in a previous review (Sangiorgio et al., 2021b), many feeding substrates have been studied, alone or in a mixture in different proportions:

- several fresh plant materials and fruit matrices, such as cabbages, carrots, oranges, apricots (Liu et al., 2020; Riudavets et al., 2020);
- various spent mushroom substrates such as those of *Pleurotus eryngii*, *Flammulina velutipes* and *Lentinula edodes* (Kim et al., 2014; Li et al., 2020);
- by-products of alcoholic beverage production, such as distillers dried grain, brewer's spent grain and beer yeast (Mattioli et al., 2021; Melis et al., 2019; Zhang et al., 2019);
- fruit and vegetable residues, such as peels of watermelon, banana, potato, carrot pomace, and beet molasses (Oonincx et al., 2015; Rovai et al., 2021; Tan et al., 2018);
- by-products from vegetable oil extraction, i.e. soybean meal, rapeseed meal, olive pomace (Rumbos et al., 2020; Ruschioni et al., 2020; Zhang et al., 2019);
- leftovers of bread and cookies (Mancini et al., 2019; Mattioli et al., 2021);
- cereal substrates (flour, non-flour and mill by-products) and legume flours (Rumbos et al., 2020; Tan et al., 2018);
- seed cleaning process of cereals and legumes (Riudavets et al., 2020; Rumbos et al., 2021);
- crop residues rich in lignocellulose, such as maize stover, rice bran and husk, straw of rice, corn and wheat (Stull et al., 2019; Yang et al., 2019b);
- various wastes of animal origin, such as hatchery waste, fish discards and even cattle and horse manure (Harsányi et al., 2020; Riudavets et al., 2020; Romero-Lorente et al., 2022).

Overall, the results of these studies indicate that larvae fed on nutrient-poor substrates show a reduced protein content but a higher fat fraction (Harsányi et al., 2020). In addition, high-protein diets lead to shorter development times and higher larval survival (Oonincx et al., 2015). However, several research studies have shown that TM can exploit the protein fraction of low protein substrates such as maize stover, concentrating it in its body biomass (Stull et al., 2019). Some plant by-products have a high level of defensive chemicals that can make them resistant to bioconversion based on insects; this is the case of wine and olive pomace, coffee and chocolate residues (Ruschioni et al., 2020). In these cases, selective breeding can be an effective tool to have adapted insects that, by detoxifying these chemicals, can feed on this kind of by-product (Jensen et al., 2017). Unfortunately, selective breeding focused on more efficient food waste reduction is still in its infancy and requires more funding and research.

Another way is to carry out successive conversion steps by combining two insects with different eating habits, such as TM and *Hermetia illucens* (black soldier fly), in a kind of multi-insect cascading biorefinery (Fig. 2). Pre-digestion of lignocellulosic-rich substrates with TM improved growth performance in the black soldier fly and led to a higher biomass production rate (Wang et al., 2017).

As pointed out by Derler et al. (2021), it is necessary to investigate the relationship between the protein content of the substrates and the growth performance/nutritional profile of TM and proceed with the standardization of TM rearing conditions to have comparable results. However, using FLW to make TM farming more sustainable has several limitations.

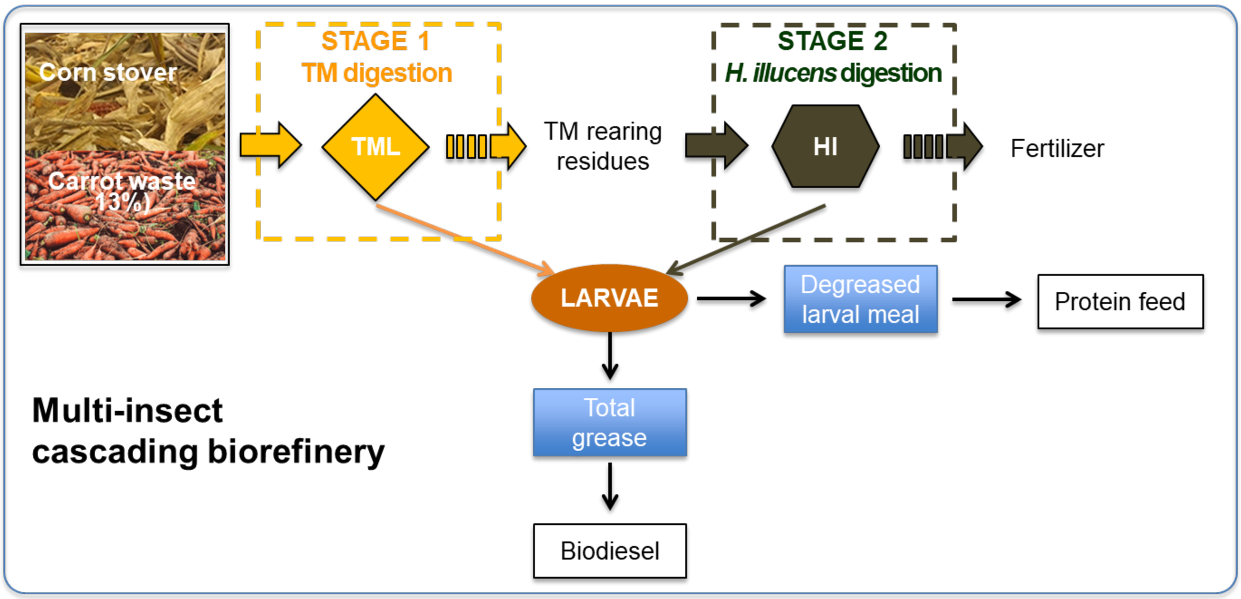


Fig. 2. Multi-insect cascading biorefinery (adapted from Wang et al., 2017)

Agro-industrial waste must not be affected by biological and chemical contaminations (e.g. pathogens, toxins, heavy metals). FLW must be low priced and of good quality. There must be no competition for the use of FLW between TM rearing and those of other animals. Finally, it is necessary to consider the seasonality of some agro-residues, together with the logistics and conservation systems for their management (Sangiorgio et al., 2021b)

Nevertheless, in the face of various limitations, there are various relevant advantages and opportunities in the approach of FLW valorization using TM. Agro-industrial waste is in large quantities and varieties. There is a large basin whence to select the optimal diet for TM growth. Furthermore, the peculiar characteristics of TM rearing allow its location close to the waste producer to reduce the costs of logistics and storage systems (Mancini et al., 2019).

Finally, the regulatory opening to TM-based products as novel food and as feed for pigs and poultry will increase the demand for TML production. Consequently, the research for beneficial and alternative feeding substrates for TM will grow.

3. Plastic degradation

The problem caused by oil-based plastics when they become waste is, as we all know, enormous. Regardless of these plastics use "in life" (most of them used for packaging), we observe that more than half (5 billion tons) of the plastics produced since 1950 by 2017 (no less than 9.2 billion tons) has become a waste that persists and exists on our planet (Plastic Atlas, 2019).

The need for new strategies to face the global plastic pollution concern is one of the most pressing problems for our society. In this perspective, entomoremediation, i.e. the use of insects for plastics degradation, has opened new opportunities to solve the problem of plastic pollution (Bulak et al., 2021).

In this article, we mainly review the scientific literature of the last few years on this topic based on a thorough previous work (Sangiorgio et al., 2021a). Several insects are capable of decomposing resistant lignocellulosic matrices (e.g. cardboard) and many plastics, such as polystyrene (PS), polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC). Among these, TM is an effective solution for the bioconversion of plastics due to its intestinal microbiota, as demonstrated for the first time by Yang et al. in 2015. These authors established the role of the intestinal microbiota and a tight relationship between the bacterial strains present in the gut of larvae and their ability to degrade plastics. The biodegradation and subsequent mineralization of plastics occur starting from their chewing by the larvae. The shredded plastic then comes into contact with intestinal microorganisms and extracellular enzymes housed in the larval gut. This contact causes the decomposition of the long-chain molecules into lower molecular weight metabolites (Yang et al., 2015). TM can carry out plastic biodegradation through biodeterioration, bio-fragmentation, assimilation, and mineralization stages. The activity of its intestinal flora is confirmed by a recent review article on the currently available microbial technologies for degrading different types of plastics (Jadaun et al., 2022).

There is a mutually beneficial symbiotic relationship between the plastic-eating insect larvae and their intestinal microorganisms. To this aim, the larvae of various insects, including TM, were compared to determine their feeding capacities and survival rates, analyze the changes in the characteristics of their productions and determine the changes in the intestinal microbiota. According to this research, feeding on PS can change gut microbiota, causing the enrichment of the microorganisms responsible for plastic degradation. For TM, *Enterococcus*, *Enterobacteriaceae*, *Escherichia-Shigella*, and *Lactococcus* were the main responsible (Jiang et al., 2021).

Bulak et al. (2021) confirmed the ability of TM to degrade plastics (e.g. PS, two types of PU and PE), which, after 58 days of testing, showed a mass reduction efficiency of 46.5%, 41.0%, 53.2% and 69.7%, respectively (with a dose of 0.0052 g/larva for each type of plastic). According to these authors, also the adult stage of the insect (imago), in addition to the larva, can "eat" plastic (Bulak et al., 2021). However, the research by Palmer et al. (2022) showed that larvae are 50 times more capable of digesting expanded polystyrene (EPS) than adults. The same authors also highlighted how the environmental farming conditions represent a decisive factor influencing the degradation potential of TM. This potential is indeed conditioned by the type of farming substrate, the plastic pre-treatments and any additional nutrients eventually provided (Palmer et al., 2022).

TM's ability to degrade plastics has been extended to different polymers: rubber of tires (Aboelkheir et al., 2019), powder from fire extinguishers (Brandon et al., 2020), plastic waste present in electrical and electronic equipment. In this last case, TM proved to be less performing than G. mellonella larvae in degrading polymers (Zhu et al., 2022). Rigid PVC microplastic powders, used as the sole diet, were also degraded by TML, which reduced polymer weight, number, and size (33.4%, 32.8%, and 36.4%, respectively). Good depolymerization but limited mineralization of PVC was also observed. About 34.6% of residual PVC polymer and a small fraction of chloride (only about 2.9% of ingested PVC) were found in the excretion (Peng et al. 2020). Poor differences in the plastic-degrading ability of TM have also been observed concerning the geographic origin for PS (Yang et al., 2018) and confirmed for PS and low-density polyethylene (LDPE), but not for polyvinyl chloride (PVC), which is less easy to digest (Wu et al., 2019).

Several studies have provided a counter-proof of the action performed by intestinal microorganisms through the use of antibiotics (by suppression tests mainly with gentamicin) that have inhibited the degradation capacity (Yang et al., 2021). In this perspective, the study carried out by Tsochatzis et al. (2021) compared the adaptation of the intestinal microbiota of TM in response to different dietary strategies and the formation of degradation compounds (monomers, oligomers). Using diets with different bran/PS content (ratios 4/1 and 20/1), it was shown that the diet with a low bran/PS ratio leads to better results from the point of view of plastic degradation (Tsochatzis et al., 2021). Similarly, the addition of wheat bran to the diet of TM increases its degradation capacity of plastic (Jaduan et al., 2022). Another observed effect is the larvae body-weight loss when fed for a long time with only plastic.

If the plastic-based meal is supplemented with conventional food for larvae (bran, usually), a marked improvement in the degradation performance of plastics is observed: insects, thanks to the nutritional supply guaranteed by the food, attack plastics better. Obviously, by increasing too much the food/plastic ratio, the effect is lost (Urbanek et al., 2020; Wu et al., 2019). Gan et al. (2021) have reached similar conclusions. They confirm that the addition of conventional food to larvae diets causes an improvement rather than an antagonism in the PS degradation. Moreover, their frass can be used as a fertiliser in agriculture. Finally, the larvae fed on these substrates can be used for food and feed (mainly poultry and fish). However, these researchers question the use of insects is quite effective for the degradation of plastics and whether this can be considered a scalable natural solution (Gan et al., 2021). In their research on the polylactide (PLA) biodegradation using TM, Peng et al. (2021) verified that PLA-bran mixtures (10%, 20%, 30% and 50% PLA, w/w) lead to higher survival and lower cannibalism rates. Based on the results of their study and the literature data, the authors proposed a sustainable approach for PLA. The production comes from biological sources (agriculture), the waste PLA supports the production of proteins (TML) and TM rearing used for PLA demolition produces residues (frass) to exploit as a soil fertilizer for agriculture (Peng et al., 2021). Further authors support this approach (Lou et al., 2021).

From this observation derives an opportunity for the closure of the bioeconomic circle: to use FLW as co-feeding of plastics. This possibility can make the process more favorable and achieve two goals, i.e. the valorization of waste and the degradation of plastic (Sangiorgio et al., 2021a). The possibility of using TM for the bioremediation of plastics presents some critical issues. The first fact negatively affecting this possibility is the excessive costs to raise a large number of insects needed for the scaling-up: considering a degradative capacity of 0.22 mg of PE/larva/day, it takes 10 tonnes of larvae to treat 1 tonne of plastic (Billen et al., 2020). Khan et al. (2021) investigate the functionality of TM plastic degradation using much lower degradation rates than others researchers and not considering other compounds (as proteins) produced from insect farming. Despite a positive evaluation they asserted, Khan et al. state that, for effective exploitation of plastic waste, it is necessary a fast conversion of plastic into biomass and by-products, and the absence of microplastics or contaminants into the biomass, not to have environmental and safety problems (Khan et al., 2021). The justified concern about the safety of products derived from insects fed with plastics is, indeed, a problem to be explored. In this perspective, Zielińska et al. (2021) have highlighted that the consumption and degradation of polystyrene do not seem to influence the state of health of insects. However, at the same time, they admit the need for further analyses to confirm the absence of toxicity in the plastic degrading insects and so allow their use safe in animal or human nutrition.

It is now recognized that the key to plastic biodegradation is the larvae intestinal microbiome. This potential needs to be exploited to upgrade it to full-scale use. For this reason, further research is needed to replicate intestinal processes and the conditions in which they occur to fully understand the synergistic actions between the digestive system of larvae and microbial metabolism and, finally, better characterise the enzymatic systems involved in the biodegradation of plastic. These results could inspire a new biotechnological approach to solve the problem of plastic waste and microplastic pollution (Pivato et al., 2022).

4. PHA recovery

Some microorganisms (e.g. *Aeromonas, Azotobacter, Cupriavidus, Clostridium, Methylobacterium, Ralstonia, Pseudomonas, Syntrophomonas*, etc.) can produce PHA as an energy reserve, mainly when they suffer growth limitations (Khan et al., 2021). PHA represents a group of natural biodegradable polyesters used for degradable bioplastics production to replace many petroleum-based plastics. The global PHA market value has been estimated at $ 215.2 million in 2020 and $ 327.3 million by 2026 (360 Research Reports, 2020). This review examines the TM contribution to PHA recovery.

The use of PHA for bioplastics production has an effective potential for further development, but some elements limit their use. Among these, one of the main limitations is the high cost of production, particularly for the lysis of the microbial cells that synthesise it and the subsequent recovery from the matrix (Li et al., 2016). Usually, the recovery of PHA is carried out through the use of solvents (chloroform, acetone, methylene chloride) or with enzymatic digestion, with heavy environmental and safety costs (Bhola et al., 2021; Ong et al., 2018a).

The use of small animals has been proved to avoid these systems. Small animals feed on cells containing freeze-dried PHA, allowing their intestine system to digest the cells and release the PHA granules with their faeces (Murugan et al., 2016). The animal model commonly used is the murine one. In recent years, insects have been preferred to rats, as they perform the same functions but require minimal resources and space and show breeding facilitation. Insects are currently used as a biorefinery for PHA recovery principally where highly pure PHA is not required (Ong et al. 2018b). In particular, the PHA recovery by TML is considered one of the best available techniques.

Even the bacterial lysis method with bacteriophages, frequently used to free PHA from cellular deposition, reveals strong criticalities compared to the use of rats or TM (Kourmentza et al., 2017). In fact, compared to bacteriophage-mediated lysis, the digestive system of TM is considered more efficient, both for ecology and for economy, downstream strategy (Haddadi et al., 2019). The comparison of the qualitative characteristics of the PHA granules obtained from TM's biological recovery with those produced with conventional methods - carried out with TEM and SEM micrographic techniques- reveals that the PHA TM-produced granules retain their sphericity and morphological traits (Ong et al. 2018a). These researchers have also shown that bacterial cells are easily consumed by TML. PHA recovery by TM fed with freeze-dried *Cupriavidus necator* cells appears the best modality since TM larvae digest cells but not PHA granules. A simple treatment with water, detergent and heat is sufficient for the purification of the PHA granules from the excretion. The entire process does not cause the loss or the morphological deformations of the molecules, as demonstrated by electron microscopy and dynamic light scattering measurements (Murugan et al., 2016).

Supplementing agri-food by-products can increase the sustainability of the TM biorefinery system for PHA recovery. Moreover, if the PHA-producing microorganisms feed on FLW, they turn a problem into a resource. Once freeze-dried, microorganisms can become a single-cell protein source and feed for TML, which, in turn, can recover PHA and release it in their frass (360 Research Reports, 2020; Zainab-L and Sudesh, 2019). In addition to the release of PHA and providing protein biomass, the larvae produce also waste from their rearing (frass, chitin, etc.) with a very high value and different applications. The conceptual scheme of the entire circle is shown in Fig. 3 (Sangiorgio et al., 2021).

In this circular bioeconomy perspective, there are a lot of wastes that can be a carbon source for PHA-producing microorganisms. Some recent works highlight how the diversity of by-products usable for the nourishment of microorganisms is very vast, including oils and serums from different matrices (Dutt Tripathi et al., 2021; Kalia et al., 2021; Surendran et al., 2020). The efficacy of the process and the ability of TM to extract PHA from various types of bacterial cells has been confirmed using *Pseudomonas mendocin* grown for 72 hours in a medium containing liquid biodiesel waste (2% v/v). The recovered PHA by TM showed high purity and higher molecular weight than that recovered by conventional extraction with chloroform (Chee et al., 2019). Another line of research is the study of the efficiency of the recovery system using TM to reduce production costs, e.g. improving TM's consumption of PHA-containing cells. Zainab-L and Sudesh (2019) have proposed a simple washing method to reduce the level of mineral salts (deriving from the culture medium) in the lyophilised cells, thus increasing their palatability for TM. Consequently, the quantity of PHA recoverable in the frass increases with a simultaneous increase in the protein fraction (79%) and a reduction in the fat content (8.3%) of the larvae (Zainab-L and Sudesh, 2019).

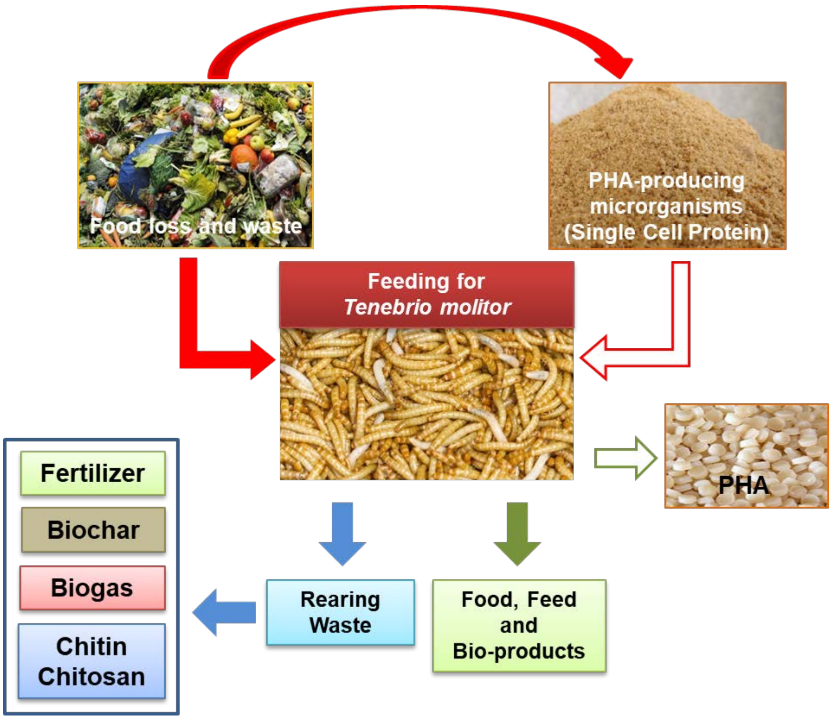


Fig. 3. TM in a circular strategy for PHA recovery, food/feed production, FLW and rearing waste valorization

5. Valorization of TM rearing waste

The TM rearing waste includes frass, dead insects, exuviae, and uneaten feed residues, commonly destined for incineration or landfill disposal. Nevertheless, to start a TM large-scale production, appropriate strategies should be made for the valorization of insect waste (Wang et al., 2017). TM frass (insect faeces) can be upcycled as fertilizing products (e.g., organic fertilizer, compost material, or soil improver) that could replace nitrogen- and phosphorus-based fertilizers obtained from conventional processes (e.g., mining and fossil-based processes). TM frass can be also converted into biogas and biochar. Instead, the TM exuviae can be exploited to obtain chitin and chitosan (Shin et al., 2019).

The global demand for products obtained from insect rearing waste is predicted to increase in the coming decades. The global markets for alternative fertilisers, chitin, and chitosan should reach, respectively, $14.7, $2.48, and $21.4 billion by 2027 from $ 4.5, $ 1.5, and $6.8 billion in 2019, at a compound annual growth rate (CAGR) of 14.1%, 11,3%, and 24,7% from 2020 to 2027 (Sangiorgio et al., 2021b). Therefore, setting up a large range of products from TM rearing waste can create new value chains and employment opportunities.

5.1. Potential TM frass exploiment as biofertiliser

It is possible to obtain 4 g of insect biomass and 180 g of frass and residues, respectively, from TML fed with 220 g of food. The volume of frass and residues is thus more than 40 times that of the produced insects (Poveda, 2021; Wang et al., 2017). In the perspective of large-scale production of TM, it is, therefore, necessary to valorize the large volume of insect frass produced. TM frass includes valuable macro-and micronutrients, such as N, P, K, C, S, Ca, Mn, Fe, Mo, and Mg, needed by plants for their growth. Huai et al. (2003) reported that TM frass use as an organic fertilizer increases the seed weight of beans (*Phaseolus vulgaris*) by 18%. Li et al. (2013) highlighted how aqueous extracts produced by using TM frass increase the germination of wheat (*Triticum aestivum*) seeds by 4%.

A recent study on chard plants (*Beta vulgaris* var. *cicla*) by Poveda et al. (2019) showed that the TM frass application as biofertiliser increases in these plants the chlorophyll content, the fresh weight, the length of the aerial part, and the width of the basal part of the stem. Research by Poveda et al. (2019) is the first study to report the potential use of TM frass to induce plant resistance to abiotic stresses, such as salinity, drought, and flooding, partly due to the presence of microbes acting as plant growth-promoting (PGP).

PGP-microbes exhibited several multifunctional abilities (Pattnaik et al., 2021; Yadav et al., 2017):

- N_2-fixation, and solubilization of micronutrients (phosphorus, potassium, and zinc);
- production of siderophores, phytohormone, enzymes, antagonistic substances, antibiotics, auxin, and gibberellins;
- secretion of exopolysaccharides (EPS), volatile compounds, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase;
- maintenance of osmolytes and antioxidants;
- regulation of stress-responsive genes etc.

The microbiome present in TM excrement includes communities of fungi, primarily dominated by *Ascomycota*, and bacteria consisting mainly of *Firmicutes,* followed by *Proteobacteria*. The predominantly present fungi family belongs to the *Aspergillaceae*. As regards bacteria, *Streptococcaceae, Clostridiaceae, and Bacillaceae* families are prevalent. In TM frass, *Aspergillus* represents the most abundant fungi genus, while the *Bacillus*, *Lactococcus*, and *Clostridium* are the most common bacterial genera found (Poveda et al., 2019).

Several research studies have reported the beneficial effect of the *Aspergillus* genus on plants. This genus of fungi showed good phosphate solubilising ability (Bhavsar et al., 2008; Pradhan and Sukla, 2006; Richardson et al., 2002) and great potential as a biocontrol agent (Soliman et al., 2012). Among the bacteria content in TM frass, also *Bacillus* can act as a biocontrol agent (Balabel et al., 2013; Borriss, 2015; Grosu et al., 2015), as well as promote seed germination (Widnyana and Javandira, 2016), root development (Aziz et al., 2015), nutrient assimilation (Shi et al., 2014), and degrade toxic waste in soils (El-Helow et al., 2013). Some strains of the genus *Lactococcus* can mineralize and either solubilise inorganic and organic phosphate sources: such as calcium phosphate, aluminium phosphate, rock phosphate, and phytate. Furthermore, the lactic acid produced by *Lactococcus* can perform an antimicrobial activity, resulting in positive effects on plant growth indirectly (de Lacerda et al., 2016). Bacteria of the genus *Clostridium* are classified as PGP due mainly to their ability to nitrogen-fixing and gibberellin-producing (Febri Doni et al., 2014).

Moreover, some scientific research performed on insects other than TM (Poveda et al., 2019; Tanga et al., 2022) have concluded that the use of insect frass as organic fertilizer can:

- contribute nutrients to the soil, mainly nitrogen, easily assimilated by plant tissues;
- add biomolecules and PGP-microbes;
- increase tolerance to abiotic stresses and resistance to pathogens and pests thanks to different compounds and microorganisms contained in insect frass.

Then, the application of organic fertilisers instead of chemical ones can represent an environmentally sound long-term approach to sustainable agriculture (Poveda et al., 2021).

Currently, the insect as a fertilizer is not clearly defined in Regulation (EC) No 1069/2009 of the European Parliament and the Council laying down health rules on animal by-products and derived products not intended for humans, even in the consolidated version of June 2019. As a result, some EU countries classify insect frass generically as manure, allowing its use as organic fertilizer after heat treatment. By contrast, other EU countries think of insect frass as a "category 2 material", different from manure, as it does not consist only of faeces. They require previous pressure sterilization before its marketing.

In both cases, the treatments carried out on insect frass cause the loss of microorganisms supporting plant health. Therefore, it is essential to establish suitable treatment processes capable of preserving the microbiological properties of insect frass (IPIFF, 2019).

5.2. Other potential TM frass uses

Besides being used as an organic fertilizer, TM frass can also be converted into biogas - specifically biomethane - via a mesophilic anaerobic digestion process. Research studies by Bulak et al. (2020) suggested that the biomethane potentials obtained from insects rearing waste (*Hermetia illucens*, *Tenebrio molitor* and *Gryllus* spp.) are like those obtained from the most used substrates for anaerobic digestion: mink, cattle and poultry manure, fruit and vegetable waste, ryegrass, switchgrass, wheat, and sewage sludge. Thus, anaerobic digestion can be considered a new method to valorize TM frass.

Finally, other research studies have suggested another method for exploiting TM frass, such as the elaboration of biochar via an insect waste pyrolysis process. Then, biochar can remove heavy metals, including Pb (II), Cd (II), Cu (II), Zn (II) and Cr (VI) (Yang et al. 2019b).

5.3. Chitin and chitosan from insects

Chitin is an inert macromolecule composed mainly of repeating N-acetyl-D-glucosamine units $(C_8H_{13}O_5)_n$. These units are linked together by β-(1,4)-glycosidic bonds (GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose). Its estimated annual production is approximately 10^{10}–10^{12} tons (Ahmed et al., 2016; Han et al. 2020; Li et al. 2019; Zainol Abidin et al., 2020), thus representing the second most abundant natural biomass after the cellulose. From a process of chitin N-deacetylation, it is generally possible to produce chitosan, a copolymer composed of β-(1→4)-linked 2-acetamido-2-deoxy-d-glucopyranose and 2-amino-2-deoxy-d-glucopyranose units (Ahmed et al., 2016).

The chitosan, discovered by Charles Rouget physiologist in 1859, is the primary chitin derivative (Maddaloni et al., 2020). Generally, three routes can be used to recover chitin and obtain chitosan: chemical, biological, and green (or physical) (Fig. 4).

Chitin and chitosan are of great commercial interest thanks to their significant characteristics, such as biocompatibility, biodegradability, low toxicity and allergenicity (Jiang et al., 2020; Maleki and Milani, 2020), and biologiocal activities, such as anti-inflammatory, antioxidant, antimicrobial, antitumor, hypolipidemic, hypocholesterolemic, anticoagulant activities etc. (Chiu et al., 2019; Kim, 2018). These macromolecules are suitable for applications in numerous fields: in agriculture, chemistry and agrochemistry, in the food, medical, pharmaceutical, cosmetic, textile and paper industries etc. (Errico et al., 2022).

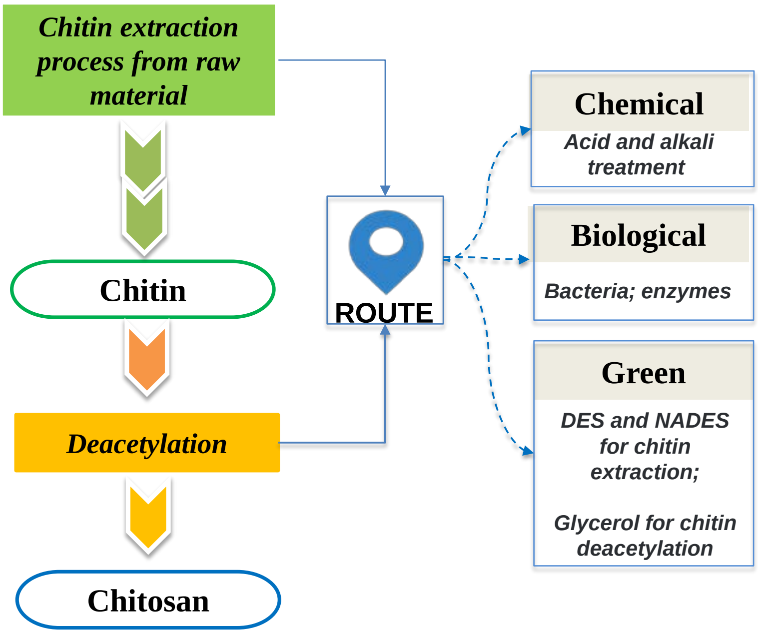


Fig. 4. Recovery processes of chitin and chitosan

The shells of marine crustaceans, such as lobsters, crabs, shrimp, krill, and crayfishes, are the primary commercial sources of chitin and chitosan. The chitin content into these animals exoskeleton is 15-40%, even if it varies widely according to the species used (quality and freshness of the shell) and the season (Morin-Crini et al., 2019). Various fungal phyla, such as Basidiomycota, Ascomycota, and Zygomycota, contain 1-15% chitin in the cell wall and represent the second chitin source (Yang et al., 2019a). Currently, insects represent a research area of considerable interest as an alternative and promising source of chitin. They produce, on average, 10-15% chitin (Costa-Neto et al., 2016).

The insects' chitin acts as a support material for: - fibrous exoskeleton cuticles; - head capsule, trachea, foregut, hindgut; - the peritrophic membrane lining the midgut lumen. (Yang et al., 2019c). It also protects insects from food abrasion and external invasion. Several scientific studies are underway relating to the extraction of chitin, and the consequent production of chitosan, from insects belonging to many different orders: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Odonata, Orthoptera, etc. Insects show several advantages over crustaceans:

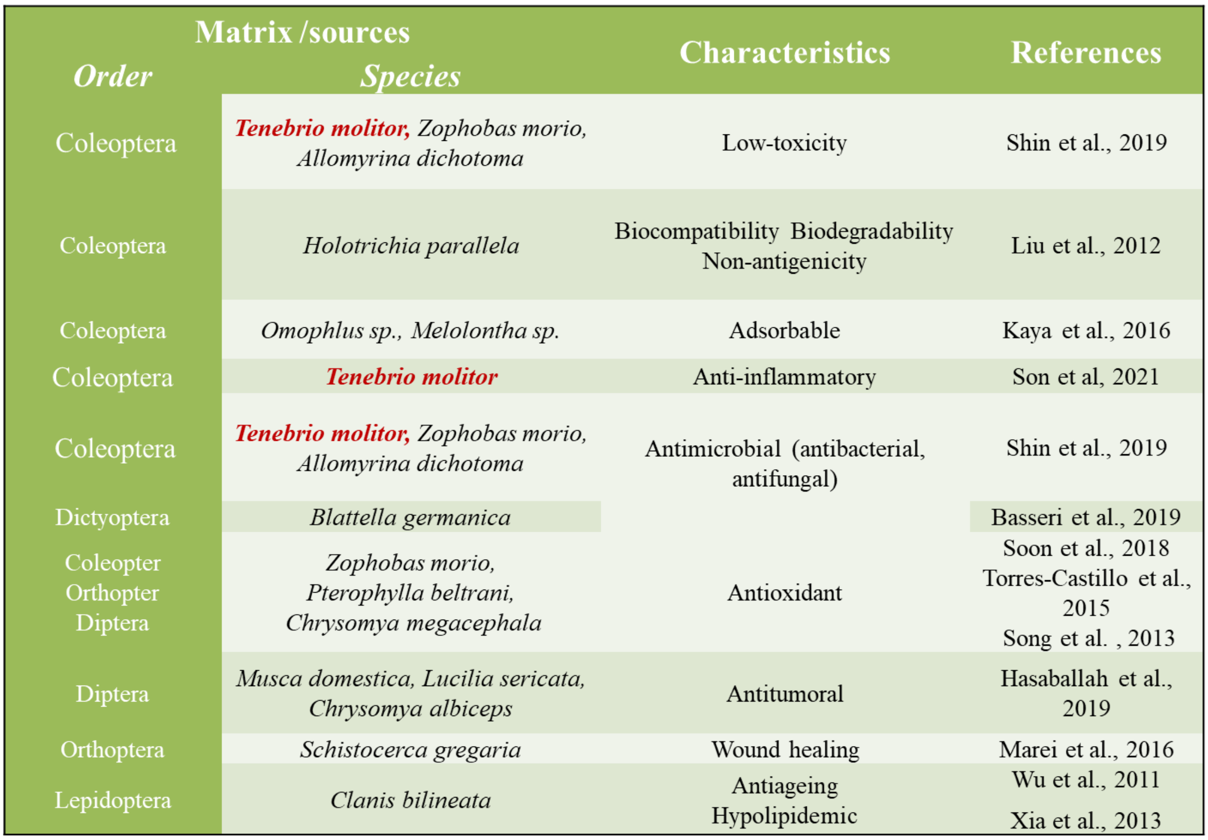
- they are very numerous as they represent approximately 80% of the world's species (Zainol Abidin et al., 2020);
- their supply is not subject to seasonality;
- their fertility and reproductive rate are high. For this reason, insects can easily be rear.
- insect rearing facilities have been made worldwide;
- insect chitin contains less than 10% inorganic material comparing crustacean shells (20% –40%). Therefore, its extraction can be performed via a more ecological, economic, and sustainable process (Hahn et al., 2020);

Figure 5 shows the characteristics of chitin and chitosan obtained from different insects. TM chitin and chitosan have low toxicity, antimicrobial (against fungi, gram-positive and gram-negative bacteria), and anti-inflammatory properties (Shin et al., 2019; Son et al., 2021). Experimental studies by Shin et al. (2019) showed for the first time that TM chitosan has antimicrobial activity against pathogenic bacteria, such as *S. aureus*, *B. cereus*, *L. monocytogenes*, and *E. coli*. Several works of literature analyse the action mechanism of chitosan against microorganisms, as shown in Fig. 6 (Li and Zhuang, 2020; Qin et al., 2010; Wu et al., 2016).

In addition, research performed by Son et al. (2021) showed excellent TM chitosan anti-inflammatory effects in the lipopolysaccharide (LPS)-induced murine macrophage cell line. Other research studies attributed the anti-inflammatory effect to also peptides, proteins, and unsaponifiable matter of the oils from TM, and not only chitosan (Chang et al., 2019; Chou et al., 2003; Son et al., 2020). Therefore, TM chitosan can be exploited in different fields, such as in medicine, in industries of textiles and food preservation, and others. Compared to other insects, TM waste can be considered a better resource for chitin and chitosan recovery thanks to the stable supply of raw materials and low cost (El Knidri et al., 2019).

6. Conclusions

In a circular economic perspective, insects such as the mealworm *Tenebrio molitor* (TM) are valid alternatives for valorizing and reducing FLW and converting them into high-value products. At the same time, fertilizers for crops and chitin/chitosan for biomaterials can be obtained by using TM rearing waste (frass and exuviae).



Matrix /sources		Characteristics	References
Order	*Species*		
Coleoptera	***Tenebrio molitor,*** *Zophobas morio, Allomyrina dichotoma*	Low-toxicity	Shin et al., 2019
Coleoptera	*Holotrichia parallela*	Biocompatibility Biodegradability Non-antigenicity	Liu et al., 2012
Coleoptera	*Omophlus sp., Melolontha sp.*	Adsorbable	Kaya et al., 2016
Coleoptera	***Tenebrio molitor***	Anti-inflammatory	Son et al, 2021
Coleoptera	***Tenebrio molitor,*** *Zophobas morio, Allomyrina dichotoma*	Antimicrobial (antibacterial, antifungal)	Shin et al., 2019
Dictyoptera	*Blattella germanica*		Basseri et al., 2019
Coleopter Orthopter Diptera	*Zophobas morio,* *Pterophylla beltrani,* *Chrysomya megacephala*	Antioxidant	Soon et al., 2018 Torres-Castillo et al., 2015 Song et al. , 2013
Diptera	*Musca domestica, Lucilia sericata, Chrysomya albiceps*	Antitumoral	Hasaballah et al., 2019
Orthoptera	*Schistocerca gregaria*	Wound healing	Marei et al., 2016
Lepidoptera	*Clanis bilineata*	Antiageing Hypolipidemic	Wu et al., 2011 Xia et al., 2013

Fig. 5. Characteristics of chitin and chitosan from different insects

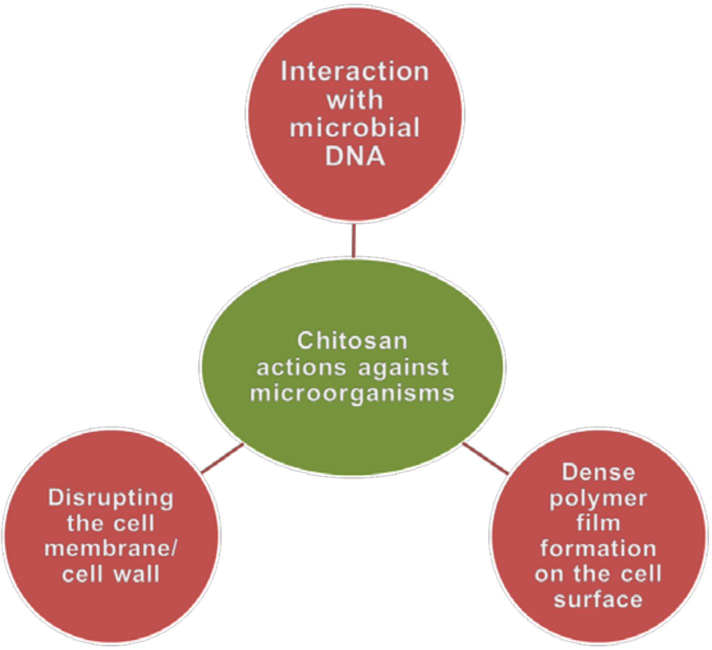


Fig. 6. Some action mechanisms of chitosan against microorganisms

The problem of plastic degradation is undoubtedly strongly much felt, and public authorities are becoming increasingly sensitive to the subject. The use of TM to degrade various types of plastics is becoming increasingly popular in the scientific literature. However, managing plastic pollution with TM does not seem to be a viable option. There are very critical authors about it. On the other hand, TM is still an insect with many interesting characteristics. It is, therefore, desirable to have more studies to highlight other TM capabilities, perhaps still unknown or little known, which can allow TM use in this sector, possibly also in combination with or in support of other solutions. For example, TM microbiota offers a valuable source of plastic-degrading microorganisms and might be exploited for this purpose in next future.

On the contrary, TM proves to be an effective and advantageous means to recover new biopolymers such as PHA from plastic-producing microorganisms. This fact could make it very interesting in the bioplastic sector.

However, in addition to the great potential, there are criticalities. TM must be produced on a large scale to meet protein needs and manage the problems of plastics and bioplastics. Unfortunately, TM industrial rearing is not yet economically and environmentally competitive.

A possible solution is to use FLW as a feed (or as a co-feed in case of plastic degradation) to reduce economic and environmental costs and, at the same time, to valorize worthless waste that represents a problem/cost. Closing the loops is another way: exploiting the waste from TM rearing and the wide range of bioproducts obtainable from the entire TM supply chain can give more value and make TM rearing advantageous.

Finally, the exploitation of TM-based products, in a sort of "entomo-refinery", could ensure the creation of new value chains and employment opportunities. Even if a lot has already been written about it over the years, TM is probably an insect that can still reserve many surprises and constitute a valid resource to be used in the best ways.

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