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Review On Biodegradation of Plastic Through Waxworm (Order: Lepidoptera; Family: Pyralidae)

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Abstract

Plastic is synthetic polymer. It is derived from fossil oil. It is resistant to biodegradation. Near about ninety-two percent of the plastic belong to Polyethylene (PE) and polypropylene (PP). Both of them are largely utilized in packaging Production of plastic production has increased exponentially in the last few years. Innovative and eco-friendly solutions for plastic degradation are urgently required. Ever increasing water pollution, soil pollution and closing of landfill sites problems have led to concern about plastics. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years. Awareness of the waste problem and its impact on the environment has awakened new interest in the area of degradable polymers. The interest in environmental issues is growing and there are increasing demands to develop material which do not burden the environment significantly. Biodegradation is necessary for water-soluble or water-immiscible polymers because they eventually enter streams which can neither be recycled nor incinerated. It is important to consider the microbial degradation of natural and synthetic polymers in order to understand what is necessary for biodegradation and the mechanisms involved. The waxworms metabolize polyethylene plastic films into ethylene glycol, a compound which biodegrades rapidly. This unusual ability to digest matter classically thought of as non-edible may originate with the waxworm's ability to digest beeswax. Two strains of bacteria, *Enterobacter asburiae* and *Bacillus sp*, isolated from the guts of *Plodia interpunctella* waxworms, have been shown to decompose polyethylene in laboratory testing.

Keywords: Plastic; Galleria Mellonella; Plodia Interpunctella; Waxworm

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Introduction

Waxworms are the caterpillars of insects, recognized generally as wax moths. Taxonomically, they are belonging to Class: Insecta; Order: Lepidoptera; Superfamily: Pyraloidea and Family: Pyralidae(snout moths). Two closely related species are commercially bred – the lesser wax moth (*Achroia grisella*) and the greater wax moth (*Galleria mellonella*). They belong to the tribe Galleriini in the snout moth subfamily Galleriinae. Another species whose larvae share that name is the Indian mealmoth (*Plodia interpunctella*), though this species is not available commercially. The adult moths are sometimes called "bee moths", but, particularly in apiculture, this can also refer to *Aphomia sociella*, another Galleriinae moth which also produces waxworms, but is not commercially bred. Waxworms are medium-white caterpillars with black-tipped feet and small, black or brown heads.

In the wild, they live as nest parasites in bee colonies and eat cocoons, pollen, and shed skins of bees, and chew through beeswax, thus the name. Beekeepers consider waxworms to be pests (Kluser, *et al*, 2010). *Galleria mellonella* (the greater wax moths) will not attack the bees directly, but feed on the wax used by the bees to build their honeycomb. Their full development to adults requires access to used brood comb or brood cell cleanings—these contain protein essential for the larvae's development, in the form of brood cocoons. The destruction of the comb will spill or contaminate stored honey and may kill bee larvae or be the cause of the spreading of honey bee diseases.

When kept in captivity, they can go a long time without eating, particularly if kept at a cool temperature. Captive waxworms are generally raised on a mixture of cereal grain, bran, and honey.

Use of Waxworms as a Food Source

Waxworms are an ideal food for many insectivorous animals and plants. These larvae are grown extensively for use as food for humans, as well as live food for terrarium pets and some pet birds, mostly due to their high fat content, their ease of breeding, and their ability to survive for weeks at low temperatures. Most commonly, they are used to feed reptiles such as bearded dragons (species in the genus *Pogona*), the neon tree dragon (*Japalura splendida)*, geckos, brown anole (*Anolis sagrei*), turtles such as the three-toed box turtle (*Terrapene carolina triunguis*), and chameleons. They can also be fed to amphibians such as *Ceratophrys* frogs, newts such as the Strauch's spotted newt (*Neurergus strauchii*), and salamanders such as axolotls. Small mammals such as the domesticated hedgehog can also be fed with waxworms, while birds such as the greater honeyguide can also appreciate the food. They can also be used as food for captive predatory insects reared in terrarium, such as assassin bugs in the genus *Platymeris*, and are also occasionally used to feed certain kinds of fish in the wild, such as bluegills (*Lepomis macrochirus*).

Use of Waxworms as Bait

Fishing bait is any substance used to attract and catch fish, e.g. on the end of a fishing hook, or inside a fish trap. Traditionally, nightcrawlers, insects, and smaller bait fish have been used for this purpose. Fishermen have also begun using plastic bait and more recently, electronic lures, to attract fish.

Studies show that natural baits like croaker and shrimp are more recognized by the fish and are more readily accepted. Which of the various techniques a fisher may choose is dictated mainly by the target species and by its habitat. Bait can be separated into two main categories: artificial baits and natural baits.

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The natural bait angler, with few exceptions, will use a common prey species of the fish as an attractant. The natural bait used may be alive or dead. Common natural baits include worms, leeches (notably bait-leech *Nephelopsis obscura*), minnows, frogs, salamanders, and insects. Natural baits are effective due to the lifelike texture, odour and colour of the bait presented. Cheese has been known to be a very successful bait due to its strong smell and light colours. Waxworms may be store-bought or raised by anglers (Weglein and Schuster, 2010). Anglers and fishing bait shops often refer to the larvae as "waxies". They are used for catching some varieties of panfish, members of the sunfish family (Centrarchidae), Green sunfish (*Lepomis cyanellus*) and can be used for shallow water fishing with the use of a lighter weight. They are also used for fishing some members of the Salmonidae family, Masu salmon (*Oncorhynchus masou*), white-spotted char (*Salvelinus leucomaenis*), and rainbow trout (*Oncorhynchus mykiss*).

Waxworms as an Alternative to Mammals in Animal Research

Waxworms can replace mammals in certain types of scientific experiments with animal testing, especially in studies examining the virulence mechanisms of bacterial and fungal pathogens (Antunes, *et al*, 2011). Waxworms prove valuable in such studies because the innate immune system of insects is strikingly similar to that of mammals (Kavanagh, *et al*, 2004). Waxworms survive well at human body temperature and are large enough in size to allow straightforward handling and accurate dosing. Additionally, the considerable cost savings when using waxworms instead of small mammals (usually mice, hamsters, or guinea pigs) allows testing throughout that is otherwise impossible. Using waxworms, it is now possible to screen large numbers of bacterial and fungal strains to identify genes involved in pathogenesis or large chemical libraries with the hope of identifying promising therapeutic compounds. The later studies have proved especially useful in identifying chemical compounds with favorable bioavailability (Aperis, *et al*, 2007).

Use of Waxworms for Biodegradation of the Plastic

Two species of waxworm, *Galleria mellonella* and *Plodia interpunctella* have both been observed eating and digesting polyethylene plastic. The waxworms metabolize polyethylene plastic films into ethylene glycol, a compound which biodegrades rapidly (Charles, et al, 2017). This unusual ability to digest matter classically thought of as non-edible may originate with the waxworm's ability to digest beeswax. Two strains of bacteria, *Enterobacter asburiae* and *Bacillus sp*, isolated from the guts of *Plodia interpunctella* waxworms, have been shown to decompose polyethylene in laboratory testing (Yang, *et al*, 2014). In a test with a 28-day incubation period of these two strains of bacteria on polyethylene films, the films' hydrophobicity decreased. In addition, damage to the films' surface with pits and cavities (0.3-0.4 μm in depth) was observed using scanning electron microscopy and atomic-force microscopy.

Placed in a polyethylene shopping bag, approximately 100 *Galleria mellonella* waxworms consumed almost 0.1 gram (0.0032 ounces) of the plastic over the course of 12 hours in laboratory conditions (Bombelli, *et al*, 2017).

The discovery of the plastic eating wax worms was accidental and incidental. It happened when a scientist and amateur beekeeper, Federica Bertocchini of the Institute of Biomedicine and Biotechnology of Cantabria in Spain was disgruntled at seeing one of her beehives at home infested with wax worms. She decided to clean the beehive, keeping the wax worms in a common plastic shopping bag while she went

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about her business. She had kept the plastic bag with the worms in it in another room. When she returned to the room she was surprised to see the worms crawling everywhere. The plastic bag was riddled with holes. This meant that the wax worms had chewed their way out of the plastic bags at a relatively high speed.

Fig.1: Adult and larva of Indian mealmoth, *Plodia interpunctella* (L).

This chance discovery gave birth to a properly conducted scientific research in collaboration with scientists at the University of Cambridge in England. Their aim was to determine whether these wax worms could be used to resolve the global plastic crisis, as published in the journal, Current Biology on 24th April 2017 (Paolo Bombelli, *et al*, 2017).

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Fig.2: Adult and Larvae of Indian waxworm, *Galleria mellonella* (L).

The Experiment On Biodegradation of Plastic Through Waxworms:

In the words of Bertocchini and her colleagues, about 100 wax worms had chewed their way out of a polyethylene shopping bag in around 40 minutes. The bag was significantly shredded within a span of just 12 hours. The researchers wanted to make sure whether the worms were just chewing through the plastic or actually eating it. Hence, they smashed the worms and smeared the plastic with the paste for observation. They noticed that about 13% of the plastic had disappeared after 14 hours! This led the scientists to believe that there was some compound in the digestive system of the worms that enabled the digestion of the plastic. On thorough analysis of the residual chewed up plastic bag, they also discovered ethylene glycol, the main compound in antifreeze, "confirming [polyethylene] degradation." Wax worms are commercially harvested to be used as fishing baits and are commonly found in beehives. They lay eggs in beehives. It is their natural habitat and diet that leads to their ability to break the chemical bond made up of carbon, found in beehives. Scientists are not yet sure of what exactly helps the worms. However, as stated by Paolo Bombelli from Cambridge University, "The caterpillar produces something that breaks the chemical bond, perhaps in its salivary glands or a symbiotic bacteria in its gut." It is to be noted that wax has a chemically similar structure to the wax found in beehives. These caterpillars are known to wreck havoc to the beehives by eating their combs. They live off the beehives like parasites (Yoshida, *et al*, 2016).

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Implication in Terms of Plastic Waste Solution

As stated earlier, about 100 wax worms were able to make holes in a plastic bag within 40 minutes and reduce it by 92 mg in 2 hours. In other words, the plastic-eating bacteria in the worms were capable of biodegrading plastic at a rate of 0.13 mg per day. The polyethylene plastic found in landfills takes 100 to 400 years to degrade. About 1 trillion plastic bags are consumed by people around the world annually, demanding a production of more than 45 million tons of polyethylene plastics in a year (Kluser, *et al*, 2010).

Other Similar Attempts on Degradation of Plastic

In order to understand the implications of this discovery better, we need to compare it with the search of other biological "digesters" which could enable plastic degradation in a speedy manner. For example, in 2011, a fungus was discovered with the capacity to erode polyurethane which is another kind of plastic that largely composes the trash heaps and ocean wastes around the globe. Further, in 2014, a team of researchers had discovered that bacteria within wax worms' digestive system happened to degrade polyethylene, but only after an interval of two months. As compared to this, the chance discovery by Bertocchini and the experiments conducted by her collaborators show that the current wax worms are able to speed up the biodegradation at a phenomenal speed (Maia and Nunes, 2013).

The Problem

Although there has been much hype about the plastic-eating property of wax worms, some scientists have warned that the solution of plastic waste is not as simple as it meets the eye. To begin with, these wax worms are known to devastate beehives, thus distressing bee population. *Galleria mellonella*, the wax worm species on which the current experiment was conducted, is one of the two species of wax worms that are considered to have caused damage to more than £4m worth of damage annually in the United States alone. Chances are that if they are bred in billions, the number required to make any significant dent on the plastic problem, they might harm the neighbouring ecosystem (Dickman, 1933).

The Right Way

Even though the wax worms have been found to wreck much more damage to plastic than their earlier counterparts, it is still not clear what exactly makes the biodegradation possible. The research team aims to pinpoint whether a single enzyme is responsible for this chemical process. If so, "its reproduction on a large scale using biotechnological methods should be achievable." This would also be economically more viable than breeding wax worms.

Alternatively, it is being speculated that the bacteria found in wax worms and responsible for biodegrading the plastic would prove to be the key. It would be possible to ferment the bacteria in vats, without depending on wax moth colonies. This would also prevent any damage to the ecosystem.

The Logical Indian team hails the discovery of plastic-eating wax worms and hopes that the research team is able to find a way forward and bring about a major breakthrough in solving the problem of plastic pollutant which is a cause of serious concern for environmentalists across the world!

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Conclusion

Plastic wastes accumulating in the environment are posing an ever increasing ecological threat. Plastics that are biodegradable can be considered environment friendly, they have an increasing range of potential application and are driven by the growing use of plastics in packaging. Polyethylene (PE) has been considered nonbiodegradable for decades. The discovery of the plastic eating wax worms was accidental and incidental. It happened when a scientist and amateur beekeeper, Federica Bertocchini of the Institute of Biomedicine and Biotechnology of Cantabria in Spain was disgruntled at seeing one of her beehives at home infested with wax worms. She decided to clean the beehive, keeping the wax worms in a common plastic shopping bag while she went about her business. She had kept the plastic bag with the worms in it in another room. When she returned to the room she was surprised to see the worms crawling everywhere. The plastic bag was riddled with holes. This meant that the wax worms had chewed their way out of the plastic bags at a relatively high speed.

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