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EXPLORATION OF SNAILS FROM CLASS GASTROPODA AND STUDIED ITS MICROBIOLOGICAL AND BIOCHEMICAL ASPECTS OF *MACROCHLYMUS INDICA*

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Abstract:

Introduction:*Gastropods represent a group of animals referred as snails or slugs. Around 62,000 species and 13,000 genera of gastropods that are reported throughout the world. Recently, number of species will exceed more than 150,000 species. One of the most diverse group i.e. Gastropods under phylum mollusc that are extremely varied in morphology (shape), feeding behavior, reproductive strategies, habitat range and size. They have the widest range of ecological niches of all molluscs. Gastropods are among the oldest known fossils, with their shells being found in rocks dating 540 million years ago. Most of these species that are reported till now that remained unchanged for over 350 million years.*

Objective:*In the present study, we focused on various snails that are found in fresh and marine water including terrestrial places. In continuation of these studies, our group focused on mucin protein extracted from one of these terrestrial snails i.e. Macrochlymus indica and studied its microbiological (antimicrobial) and biochemical (amino acid estimation) aspects.*

Methods:*In this study, we examined biochemical parameters (protein, Nanodrop method and amino acids profile, Bradford reagent) in mucin protein and also estimated its antimicrobial activity using bacterial and fungal strains.*

Results: *Mucin showed the presence of protein including rich amount of amino acids i.e. valine, serine and leucine. In addition, mucin at higher concentration showed its antimicrobial activity against these bacterial and fungal strains.*

Conclusion: *Overall the results of mucin showed its antimicrobial activity and this activity could be due to the presence of rich amount of protein and amino acids i.e. valine, serine and leucine.*

Key words: *Gastropods; mollusc; Macrochlymus indica; microbiological; biochemical*

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INTRODUCTION:

One of the most important group of invertebrates after arthropods in terms of species number i.e. Molluscs [1]. As per the literature, more than 110,000 known species are reported and most of them are marine. In molluscs, there is prominent feature i.e. calcareous shell that is reported in various mollusc species i.e. snails, oysters, clams, squids etc. [1,2] In contrast, molluscs are mentioned in the literature as bilaterally symmetrical, well developed excretory, digestive, respiratory and circulatory systems [3,4]. Overall, molluscs are generally characterized and showed three main body regions i.e. head-foot (sensory and locomotor part of the body); visceral mass (containing most of the organ systems) and mantle (covers the visceral mass and secretes the shell). Most of the molluscs also showed the presence of radula **(**rasping structure covered with chitinous teeth) that is required or used for feeding purpose [1-4]. Generally, mollusc are probably closest phylogenetically to the annelids. According its classification, molluscs are divided into four classes [1-4] as shown in **Table 1**.

Out of these four classes of Molluscs, Gastropods (dioecious and some forms are hermaphroditic) are included as one of the largest group belong to the phylum Mollusca (classification of invertebrate animals) and reported more than 40,000 species. Out of these species, more than 80 % of these species corresponds to living molluscs [5]. Gastropod feeding habits are totally varied extremely, although most of these species make use of radula in some aspect with respect to its feeding behavior i.e. some of them graze or feed on plankton or some of them are scavengers or detritivores or active carnivores [4-6]. Most familiar examples of gastropoda class i.e. snails and slugs that showed well developed head, eyes, tentacles (1-2 pairs) etc. [7] In contrast, gastropods are characterized on the basis of its feature i.e. torsion (results in the rotation of the visceral mass and mantle on the foot). In gastropods, torsion showed some peculiar feature i.e. wastes are expelled from the gut and nephridia near the gills. A variety of morphological and physiological adaptations have arisen to separate water used for respiration from water bearing waste products [8].

As per the literature, molluscs species under invertebrate group and used as a source of food for humans and also used for manufacturing as well as synthesizing pearls, sea silk, chemical compounds etc. [9] Most of these molluscan species also used in the form of currency in some preindustrial societies. In the literature, molluscs species also bite or sting humans and also some of them reported as agricultural pests [10]. In other words, molluscs species directly or indirectly correlated with humans. In the present study, our group focused on snails that are reported in fresh water, marine water and terrestrial areas as well. In view of this, one of these terrestrial species especially *Macrochlymus indica* are reported and determined its antimicrobial potential of mucin protein and also analyzing its protein including amino acid content.

Distribution of gastropoda (snails) in case of fresh water, marine water and terrestrial area

One of the well-known groups of invertebrates i.e. Molluscs which is associated directly or indirectly with humans since the dawn of civilization. In the literature, molluscs bodies were gathered for food and its shells were used in the form of tools, ornaments etc. one of the most peculiar species of molluscs which are able to survive or adapt in different environments (i.e. marine, freshwater, and terrestrial) [11, 12]. In phylum mollusc, one of its class i.e. Gastropoda showed diverse groups of living animals that are reported in fresh water, marine water and terrestrial areas. All these information represents long as well as rich fossil record. In other words, gastropoda (e.g. snails), unique animal group of invertebrate and these are reported in shallow-water benthic communities (marine gastropods); colonized in land environments i.e. ranging from lowlands to high mountains including humid to arid

CLASSES	FEATURES
Bivalvia (lack radula)	Hinged shell with right and left halves that covered its visceral mass. In bivalves, foot extends out between the shells and is used for locomotion e.g. mussels, clams, and oysters.
Polyplacophora	Elliptical body with a shell composed of eight plates.
Gastropoda	Visceral mass is contained in a spirally coiled shell, there is a distinct head with one or two pairs of tentacles and the foot is large and flat e.g. snails and slugs.
Cephalopoda	Prominent head with complex eyes and eight to ten tentacles surrounding the mouth. The shell may be internal or external E.g. squids and octopi.

biotopes of tropical to subarctic areas (terrestrial gastropods)[13]. The most familiar example of gastropods i.e. Snails and these are one of the earliest known types of animals in the world. Gastropods are able to adapt to a variety of living conditions and they don't require large amounts of food. According to the literature, most of the molluscan species that are reported with shells which can produce pearls but only the pearls of bivalves including some gastropods where shells are lined with nacre that are more valuable [1-4].

In gastropods, reproduction totally varied among species. Hermaphroditism is common in all species of gastropods but in case of marine species, individuals serving as either male or female during mating [14, 15]. Normally, shape shell determines the manner how gastropod mates e.g. Snails with tall shells used mounting technique while snails, flat shells often mate in a face-to-face fashion. All gastropods, however, reproduce through internal fertilization. In some instances, sperm can be stored for several months or years before being used to fertilize eggs. Mates are located through touch and the use of pheromones, or by sensing cues in the mucus trail left by terrestrial slugs. Most of gastropods species are oviparous (egg laying). Land snails (terrestrial) deposit eggs in nests dug in moist soil. Many aquatic gastropods deposit their eggs in a gelatinous mass containing as many as 40,000 individuals. During spawning season, some gastropods can lay eggs in intervals of one or two days. Others, however, lay much smaller clutches (groups of eggs) two or three times each season. More common are the "mermaid's necklaces" laid by the whelks. These long chains of egg cases can be found on beaches throughout the world. Juvenile gastropods are often called protoconchs and are shaped like the adults [14, 15]. In the present study, our group focused on different types of snails that are reported especially in fresh water, marine area and terrestrial areas as shown in **Table 1.**

Biochemical and Microbiological studies

In Phylum mollusc, Gastropoda are included and recognized as one the largest group after phylum Arthropoda in terms of species distribution. Among gastropods, nearly 35000 species of terrestrial snails (also known as Land snails) are reported in all over the world [16]. Most of these terrestrial snails are very harmful and caused economic damage to agri-horticultural crops and forestry. One of the terrestrial snail i.e. *Macrochlymus indica* is identified and collected from the garden of Vidya Pratishthan's School of Biotechnology, Baramati, Maharashtra, India. This snail is reported as pest inflicting damage to a wide arrange of vegetable and ornamental crops in India. Number of cases related to this species are reported in several states i.e. West Bengal, Orissa, Assam, Bihar, Kerala, Tamil Nadu, Delhi and Port Blair. So, terrestrial snails (also known as Land snails) are one of the important component and indicator of forest's ecosystem and ecological conditions. In India, around 1129 species are reported; out of these more than 80 % of these species that are endemic and found in Western Ghats and north –east India. The most familiar examples of terrestrial snails (i.e. *Deroceras laeve, Laevicauli salte, Macrochlymus indica*, etc.) and reported as serious pests to horticultural and agricultural systems [16, 17]. These terrestrial snails are hermaphroditic and secrete a trail of mucus from their pedal gland while traveling across a surface and it showed various biological functions. In snails, mucus contained 90-99.7% water by weight and it consists of giant protein polysaccharide complexes. This complex further classified into mucopolysaccharides and glycoproteins. According to the literature, functionality of trail mucus, including its interactions with water vapour, can therefore lead to a means of controlling the reproduction of snails and thereby limiting their impact on the environment, especially vegetable crops [17].

In the literature, mucins represents one of the major glycoprotein components of mucous that normally coats on the cell lining surface of the respiratory, digestive including and urogenital tracts and also reported in some molluscs species (e.g. *Macrochlymus indica*) and amphibians. The major role of mucus is to protect epithelial cells from infection, dehydration etc. Some of the mucin family members are reported that are varied with respect to its size, some of them are small and few of them contained a long chain of hundred amino acid residues. In this regard, we focused on certain amino acids that are reported in one of the molluscan species e.g. *Macrochlymus indica*. The results of these studies related to amino acid content (serine, valine, tyrosine, leucine) in mucin protein which is determined through Bradford reagent as shown in **Fig.1.** From these studies it is confirmed that mucin contained higher as well as rich amount of amino acid residues i.e**.** valine, serine, tyrosine and leucine as shown in **Fig.1**. Moreover, these domains are composed of tandemly repeated sequences that are totally varied in number, length and amino acid sequence from one mucin to another. In addition, protein also estimated from snail mucin through Nanodrop method as shown in **Fig.2**.

In addition, protein content of mucin extracted from *Macrochlymus indica* and it was determined through Nanodrop method and also estimated its antimicrobial activity using various gram positive (*Salmonella typhi, Bacillus subtilis*) and gram negative (*E. coli, Pseudomonas aeruginosa*) bacteria including fungal (*verticillium lecanii*) samples. Four bacterial species were used in the study and these samples were collected from soil of VSBT garden and

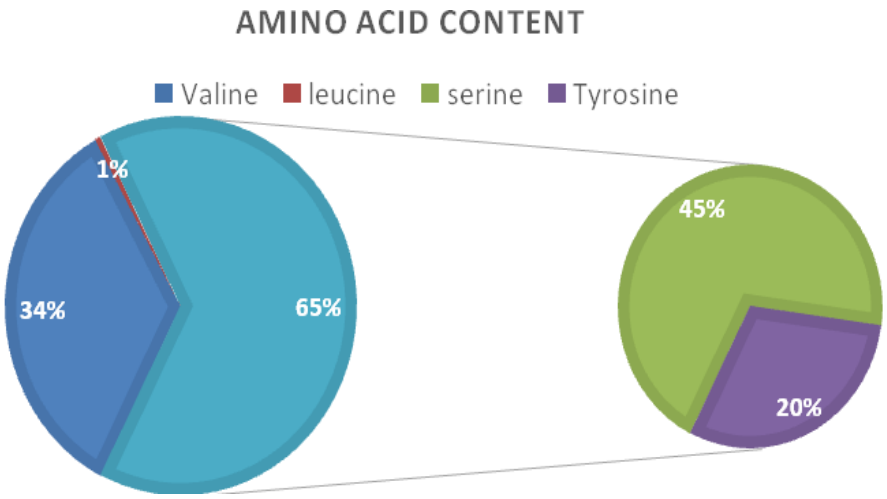

Fig.1. Amino acid content in mucin protein extracted from *Macrochlymus indica*

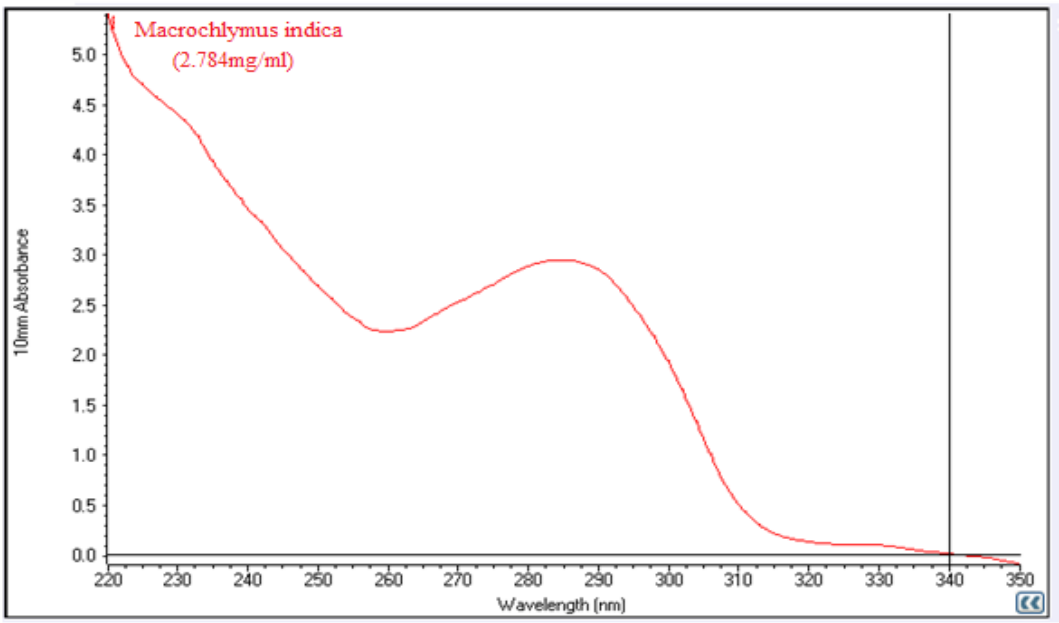

Fig.2. Estimation of protein from snail mucus extracted from *Macrochlymus indica*

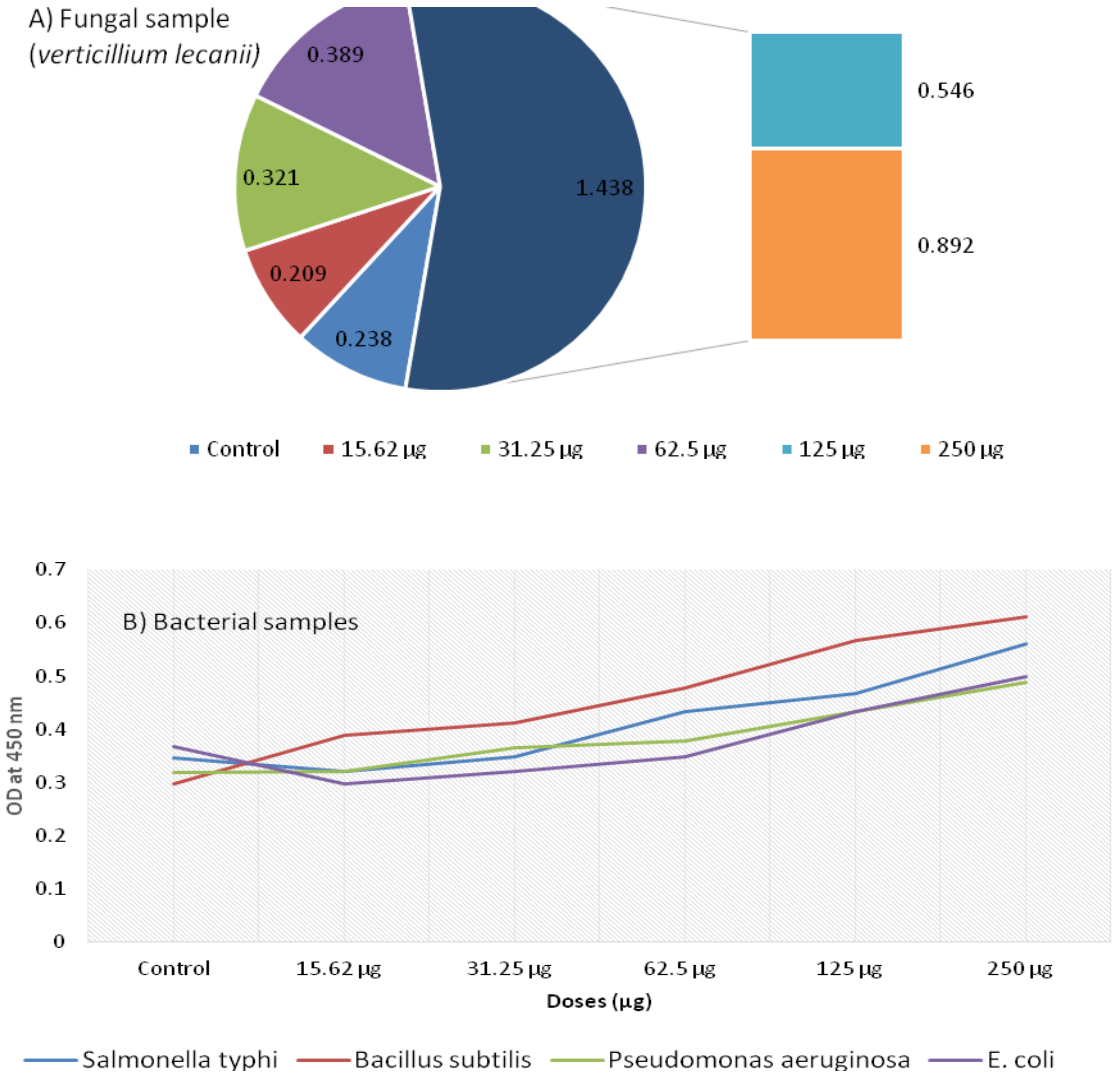

Fig.3. Detection its antimicrobial activity of mucin protein which is determined through Elisa method. A) Antifungal activity B) Antibacterial

confirmation of these samples through gram staining method. In addition, fungi samples were identified on the basis of morphological and biochemical characteristics. In this study, fungal spore strains are diluted in phosphate buffered saline to get 10^5 spores/ml. Strains of bacterial (10^5 CFU/ml) and fungal (10^5 spores/ml) were used as coating antigen in Elisa plate (overnight, 4 ºC) for estimation of antibody production against mucin protein. In other words, mucin from *Macrochlymus indica* were used for estimating its antimicrobial activity. After incubation, block Elisa plates with 1% BSA (bovine serum albumin) solution and incubate it for 30 minutes at room temperature. Thereafter, add variable concentration of mucin protein (15.62 – 250 µg) of *Macrochlymus indica* and incubate it for another 4 h at carbon dioxide incubator. After incubation, wash again with PBS and then add horse anti-serum (1:1000 dilution; 100 µl) used as secondary antibody. Incubate the plate for another 1 h at carbon dioxide incubator. After incubation, trimethyl benizidine (TMB, 100 µl) substrate was added and keep it in dark for 15 minutes and then add stop solution (1N H_2SO_4). The optical density was measured at 450 nm. The results of these studies showed that mucin protein at higher doses showed enhancement in antibody production against these bacterial strains and fungal strain (**Fig.3**). In other

words, mucin protein showed its antimicrobial activity against bacterial and fungal strains.

CONCLUSION:

In this paper, we mentioned about various snails that are reported in fresh water, marine water and terrestrial places. One of the terrestrial snail i.e. Macrochlymus indica is identified and studied its antimicrobial activity of mucin protein. This activity could be due to the presence of number of amino acids that are present and showed enhancement in antibody production against these bacterial and fungal strains. Further studies were still is in progress.

AUTHORS CONTRIBUTION

This work was carried out in collaboration between four authors. Amit Gupta and Shweta Karne designed the study, wrote the protocol and interpreted the data where Shweta Karne anchored the field study, gathered the initial data related to her M.Sc Microbiology dissertation work under Amit Gupta guidance and performed preliminary data analysis. Amit Gupta, Shweta Karne, Sanjay Kamble and Bharat Shinde managed the literature searches whereas Amit Gupta and Shweta Karne produced the initial draft. The final manuscript has been read and approved by all authors.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Table 1: MORPHOLOGICAL DISTRIBUTION OF SNAILS

SNAILS	DISTRIBUTION	STRUCTURE	FOOD	USE	DISADVANTAGES
MARINE SNAILS					
Buccinum undatum (*Buccinidae*)	North Atlantic along the coastline of North America, France and Norway.	Calcified shell (6-10 cm) in length; spiraled shell.	Carnivore (feeds on crabs, polychaete worms, bivalves etc.	Used as food.	Larval stages of parasite (*Stephanostomum baccatum*) found in the digestive gland
Turbo cornutus (Turbinidae)	Widely distributed in Mascarene basin, Philippines, Japan and china	Hard, ventricose, spiny, imperforate shell (≅ length 65 - 120 mm). Large, thick, green-gray shell with irregular incremental striae and spiral lirae; shell (5-6 whorls, turn clockwise and horny protuberances); body whorl is ventricose, somewhat bicarinate, armed about the middle with two spiral series of erect tubular spines.	It feeds on various kinds of algae.	Used as food.	None
Sinistrofulgur perversum (Busyconidae)	Southeastern (North America) and Florida. Sinistral in coiling.	Left-handed, sinistral shell.	It eats mostly bivalves.	used as food and their shells used for tools, ornaments etc.	None
Knobbed whelk (Melongenidae)	Most common whelk in South Carolina	Adult whelks are usually 13 to 23 cm in length and characterized by low knobs on the shoulder of the whorl with the aperture on the right side. Aperture coloration ranges from light orange-yellow to brick red.	carnivorous gastropods that feed on bivalves i.e. hard clams, oysters etc.	Used by humans as food in such dishes as salads (raw), burgers, fritters, and chowders.	None
Common periwinkle (Littorinidae)	Northeastern coasts of the Atlantic Ocean, including northern Spain, France, England, Scotland, Ireland, Scandinavia, and Russia	Single spiral shell that grows with their bodies protects these small snails. Body includes a fleshy foot, short tail and two antennae on the head. The cream-colored foot of the common periwinkle is divided into right and left half, which the snail moves alternately as the muscle ripples forward. Their stalked tentacles are sensory organs that are used to see and taste.	Omnivorous, grazing intertidal gastropod. It is primarily an algae grazer, but it will feed on small invertebrates such as barnacle larvae.	Used as food (high in protein, omega-3 fatty acids and low in fat).	None
FRESH WATER SNAIL					
Pomacea bridgesii (Ampullariidae)	Amazon river system (Bolivia, Brazil, Paraguay and Peru).	Shell (5 to 6 whorls; 40-50 mm wide and 45-65 mm high); characteristic of this shell i.e. square shoulders (flat at the top of the whorls) and almost 90° sutures. The shell opening (aperture) is large and oval, umbilicus is large and deep.	Dead and rotting plants and artificial foods like fish food.	Kept in aquarium as pet	None
***Clea Helena* (*Buccinidae*)**	Reported in Malaysia, Thailand, and in lake Toba on Indonesian island Sumatra	0.7-1.25 in (20-35 mm) in size. The shell (conical in shape), consists of dark brown and yellowish tan bands, leading to some people to refer to this snail as the bumble bee snail.	Carnivorous (feeds on worms and gastropods)	Used in aquarium	None
Anisus vorticulus (Planorbidae)	Distributed and reported in Poland, England, Croatia, Germany etc.	Shell horny brown, upper side slightly concave, lower side almost flat, convex whorls with distinct suture, last whorl with blunt edge in the center of the periphery, aperture elliptical and usually oblique.	Feeds on diatoms or algae	None	None

Melanoides tuberculata (Thiaridae)	Widely distributed in Egypt, Ethiopia, Libya, Namibia, Niger), South Africa and china	This species has an elongate, conical shell, which is usually light brown, marked with rust-colored spots. An operculum is present.	Feeds primarily on algae	Used as algae-eaters and substrate cleaners.	Agricultural pest species
TERRESTRIAL SNAIL					
Macrochlymus indica	Widely distributed in India, Egypt, Ethiopia, Libya, Namibia, Niger), South Africa, china	Elongated and purplish grey in color. Small right shell-lobe and left is narrowly communicated over the edge of the peristome and its basal side gives off a short tongue-like process.	Nocturnal, hidden in soil and normally feed on the leaves of beans, cabbage, cauliflower and some wild plants for food.	None	Agricultural pest species
Helix aspersa (*Helicidae)*	Widely distributed in Great Britain, Western Europe, North Africa including Egypt.	Hard, thin calcareous shell, four or five whorls. Shell is variable in color and shade but generally is dark brown, brownish golden, or chestnut with yellow stripes, flecks, or streaks (characteristically interrupted brown colour bands).The aperture is large and characteristically oblique, its margin in adults is white and reflected.	Mucus-derivated drugs used in various therapies e.g. skin abrasions and scars to cure respiratory diseases.	None	Agricultural pest species