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APPLICATION OF NEMATODE DERIVED ENZYMES IN INDUSTRIES-A REVIEW

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ABSTRACT

A nematode is a roundworm, a member of the phylum Nematoda. They are the most abundant multicellular animals on Earth, with over 28,000 known species. Nematodes can be found in almost all environments, including soil, water, plants, animals, and even the human body. There are many enzymes that have been derived from nematodes like Cellulase, Amylase, Pectinase, Lignin Digesting enzymes, Hemicellulase etc. These enzymes are important for a variety of industrial and agricultural applications. They are used to make food, paper, biofuels, and bioplastics. They are also used to improve the digestibility of plant material for livestock. These enzymes are essential for the breakdown of plant cell walls, which makes it possible to use these materials for a variety of purposes. Cellulase is used to make biofuels, such as ethanol and biodiesel. It is also used to make paper, textiles, and bioplastics. Amylase is used to make bread, beer, and other food products. It is also used to make industrial chemicals, such as glucose syrup and starch acetate. Pectinase is used to make fruit juices and wine. It is also used to make textiles and other industrial products. Lignin-digesting enzymes are used to make paper, biofuels, and bioplastics. They are also used to treat soil contamination and to improve the digestibility of plant material for livestock. Hemicellulase is used to make food, paper, and biofuels. It is also used to improve the digestibility of plant material for livestock.

Keywords: Nematodes, Enzymes, Cellulase, Amylase, Hemicellulase, Lignin Digesting Enzymes, Industry, Industry Application

NEMATODES

The most prevalent group of multicellular organisms on earth, nematodes are a less well-known category of these creatures. They are a class of multicellular, translucent, pseudocoelomate creatures that resemble threads or worms and are either free-living or parasitic on plants or animals. The Animalia kingdom's greatest phylum to date is Arthropoda. Nematodes, on the other hand, are the most prevalent species. Nematodes make up four out of every five multicellular species on our planet. Nematodes make up around 90% of all multicellular creatures on earth. [1] They make up the meio and mesofauna's most numerous phylum in terms of numbers. Nematodes are something unseen and unheard of for many of us, though. It is hypothesized that this is because of their diminutive size and propensity to hide in soil, water, plant, and animal tissues. Because they are so common, nematodes are connected to humans, domestic animals, plants, insects, and other invertebrate and vertebrate organisms. They display a variety of lifestyles, including terrestrial, aquatic (marine and freshwater), free-living, predatory, insect partners, and entomopathogenic. Compared to plant-parasitic and free-living nematodes, understanding of animal parasitic nematodes is significantly older. We were aware of animal parasite forms as early as 1500 BC. At that time, people were aware of large round worms like Ascaris lumbricoides and the infamous Guinea worm, Dracunculus medinensis, etc.[2] On the other hand, we did not have much knowledge on soil nematodes for a very long time. This is thought to be caused by the covert lifestyle these creatures lead as well as by their tiny size. Turbatrix aceti (vinegar eel), a free-living nematode, was first seen by Borellus.[3] Needham reported the first plant-parasitic nematode.[4] Systematics of nematodes was first published by Rudolphi.[5] Leidy was the first one to describe a freshwater nematode, Tobriluslongus.[6] Dujardin for the first time described a dorylaim nematode, Dorylaimus stagnalis.[7] An average of 15,000–20,000 juveniles of Anguina tritici is present in a single wheat gall. Many million individuals per m^2 in soil and bottom sediments of aquatic habitats may be present and it is not uncommon to find more than 50 species in a handful of soil. Nathan Augustus Cobb, referred to as the Father of Nematology in the United States very rightly said, "If all the matter in the universe except nematodes were swept away, our world would still be recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes."[8]

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ENZYMES

It is well known that enzymes are highly helpful in many industrial processes. Since they have a wide range of applications, there has been a major increase in market demand. Market studies on global enzyme demand have identified a number of important elements that contribute to the enormous consumer demand for enzymes. Some of them are wholly dependent on economic advancements. An enormous expansion in consumer-related industrial applications, for instance, is brought on by rising per capita wealth in emerging nations. According to a recent market report on "Global Industrial Enzymes" conducted by Freedonia in January 2018, the demand for industrial enzymes is expected to increase by 4.0% annually to $5.0 billion in 2021. Several investigators have reported the presence of the enzyme's amylase, cellulase, in homogenates of certain free-living, mycophagus, and plant-parasitic nematodes. Homogenates of Ditylenchus destructor (Fig. 1) [9], D. disacci [10] and D. triformis [10-11] yielded amylase activity but none was found in Meloidogyne incognita (Fig. 3) acrita [11] and Diplogasrter lheritieri [12]. Cellulase activity was demonstrated in D. destructor [13], D. dipsaci (Fig. 2)[13-16], D. triformis [10-11, 14], D. myceliophagus [13-14], Pratylenchus zeae [10], P. peyaetrans [15], Heterodera schachtii [14], H. trifolii (Fig. 4)[15], M. incognita acrita [11-14], M. Arenaria [14], Helicotylenchus nannus[14], Aphelencbus avenae [14], and Tylenlchulus semipenetrans[14]. Krusberg suggested that the distribution of hydrolytic enzymes might prove interesting since Turbatrix aceti, a bacterial feeder, lacks cellulase activity, while all nematodes feeding on fungi and higher plants seem to possess this enzyme. [16]

CELLULASE

Cellulase is the enzyme of industrial interest and plays a crucial role in hydrolysis of cellulose, a prime component of plant cell wall[17]. In the market for industrially significant enzymes, cellulase spans a wide range of applications, and it is ranked as the third-largest industrial enzyme globally.[18] There have been reports of possible secretory enzymes that let the nematode to go through various developmental stages of plant tissues [11]. In particular, cellulase activity has been demonstrated in juveniles from several migratory and sedentary nematodes [12, 15]. Increased cellulase activity has been reported in galls of tomato roots infested with M. javanica [19], but enzymatic tests on secretory granules of M. incognita juvenile and females do not confirm the nematode origin of this activity [20]. Endogenous production of cellulases by cyst nematodes has recently been unambiguously demonstrated by the isolation of β-1,4 endoglucanase cDNAs from G. rostochiensis and H. glycines infective Juvenile.[21] It was demonstrated that secretion through the stylet of β-1,4-endoglucanases occurs at this stage in G. rostochiensis.[21] Application of cellulases in the pulp and paper industry has increased considerably during the last decade [22]. The woody raw material is refined and ground during the mechanical pulping process, resulting in pulps with high fines, bulk, and stiffness contents. The use of cellulases in biomechanical pulping, however, led to significant energy savings (20–40%) during refinement and increases in hand-sheet strength qualities.[23-24]

Different forms of paper waste can be deinked with cellulases alone or in conjunction with xylanases. The majority of currently suggested applications utilize cellulases and hemicellulases to partially hydrolyze carbohydrate molecules in order to release ink from the fiber surface.[25] The most effective enzymes for wet processing of textiles, particularly for finishing cellulose-based textiles with the aim of enhancing hand and look, are cellulases.[26-27] Perhaps the most well-known application being researched right now is the enzymatic saccharification of lignocellulosic materials such as sugarcane bagasse, corncobs, rice straw, Prosopis juliflora, Lantana camara, switch grass, sawdust, and forest leftovers by cellulases for the manufacture of biofuel. [25, 28] Alcoholic drinks, such as beers and wines, are produced by fermentation processes that depend heavily on microbial glucanases and related polysaccharides. [28-30] The macerating enzymes complex, which includes cellulases, xylanases, and pectinases, is used to extract and clarify fruit and vegetable juices in order to maximize the production of juices.[31-32] The nectars and purees from tropical fruits including mango, peach, papaya, plum, apricot, and pears are made more stable and have a smoother texture thanks to the macerating enzymes .[33] Olives are crushed and ground in a stone or hammer mill, the ground olive paste is passed through many malaxeurs and horizontal decanters, and high-speed centrifugation is used to extract the oil. Also used in carotenoid extraction .[30] Use of cellulases along with protease and lipase in the detergents is a more recent innovation in this industry .[23] Additionally, due to its enormous demand across several industries, including the manufacture of biofuels, pulp, paper, textile, food, drinks, as well as the detergent industry, cellulase contributes around 20% of the global enzyme market .[34] Among them, the commercial production of biofuels in the future will substantially take up the need for cellulase, and this will further increase the demand for cellulose from the biofuel business .[18] The most desired product for the manufacturing of biofuels is glucose, which is produced by cellulase hydrolyzing a cellulosic substrate.[34] Cellulase enzyme used in different industries i.e., Agriculture, Bioconversion, Detergent, Fermentation, Food, Pulp and Paper, Textiles etc.[25] The hydrolysis of internal a-1,4-glycosidic connections in starch to produce low molecular weight products like glucose, maltose, and maltotriose units is catalysed by a-amylases.[35-37] Amylases, a type of industrial enzymes that account for around 25% of the global enzyme industry, are among the most significant enzymes and have significant biotech

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implications.[37-38] They come from a variety of sources, including plants, animals, and microbes. They discovered that the alpha-amylase gene, which codes for one of the essential starch-converting enzymes, is somewhat increased during the genome sequencing effort of D. destructor.[18] As one of the 13 glycosyl hydrolases, alpha-amylase may hydrolyze starch by cleaving to the alpha-1,4 glycosidic linkages of the inner (endo-) section of the amylose or amylopectin chain. The triple-copy alpha-amylase genes of D. destructor are thought to be pathogenesis-related genes that aid in the worms' ability to parasitize hosts. However, it is unknown if the alpha-amylase genes actually play a significant role during plant-parasitic nematode infection. According to speculation, D. destructor may possess special pathogenesis-related genes that enable it to parasitize starch-rich hosts. Here, we concentrated on D. destructor's multi-copy alpha-amylase genes, which produce a crucial starch-catalyzing enzyme. They previously revealed the D. destructor genome, which contained the three genes Dd_02440, Dd_11154, and Dd_13225 that encode alpha-amylase. The three genes from D. destructor were closely clustered in the phylogenetic tree, indicating that there was a specific expansion of the alpha-amylase gene in D. destructor, according to the comparative analysis of alpha-amylases from different species, which showed that the other plant parasitic nematodes, even Ditylenchus dipsaci in the same genus, harbour only one or no alpha-amylase gene. An enzyme assay was used to confirm the three alpha-amylase proteins enzymatic activity. The three alpha-amylase genes were discovered to be substantially more expressed in D. destructor post-hatching stage than they were in eggs, according to a quantitative rea-time PCR analysis.[39] The starch industry uses a-amylases most frequently for starch hydrolysis in the starch liquefaction process, which turns starch into fructose and glucose syrups.[40] The major users of enzymes in terms of volume and price are the detergent industry. The use of enzymes in detergent formulas improves the detergent's capacity to eliminate challenging stains while also making it ecologically friendly. 90% of all liquid detergents contain amylases, the second kind of enzyme employed in the creation of enzymatic detergent.[41] The most common liquid biofuel is ethanol. Starch is the most commonly utilised substrate for ethanol synthesis since it is inexpensive and a readily available raw material in most parts of the world.[42] In this process, starch must first be solubilized before going through two enzymatic stages to produce fermentable sugars. Liquification and saccharification, in which starch is transformed into sugar by an amylolytic microbe or enzymes like a-amylase, are required for the bioconversion of starch into ethanol .[43-44] Amylases are widely used in the processed food business in processes including baking, brewing, making digestive aids, making cakes, and making fruit juices and starch syrups. In the textile business, amylases are utilized in the desizing process. Before making fabric, sizing agents like starch are put to the yarn to facilitate a quick and secure weaving process. Because it is cheap, widely available in most parts of the world, and readily removeable, starch is a highly alluring size. In the textile finishing business, starch is afterwards taken out of the woven cloth using a wet process. Desizing is the process of removing the fabric's starch, which acts as a stabilizing agent to keep the warp thread from breaking when weaving. The a-amylases remove only the size and spare the fibres.[45] The purpose of using a-amylases in the pulp and paper business is to modify coated paper's starch in order to create low-viscosity, high molecular weight starch.[35] Many microbial amylases are now commercially accessible, and they have nearly entirely supplanted chemical starch hydrolysis in the starch processing business. Microorganisms' amylases have a wide range of commercial uses because they are more stable than those produced using a-amylases from plants and animals.[46] Due to their relative ease of large-scale synthesis (cheap downstream cost because they are extracellular in nature) compared to amylases from plants and animals and their usefulness in afterwards applying to industry, amylases of bacteria, fungus, and viruses are being explored more and more. [47] Alpha amylase and other enzymes, including glucoamylase and cellulose, are necessary to create fermentable sugars that may be converted to ethanol.[48] Amylase from apple was tested for its application in for the removal of fibrous stains from the fabrics and has shown potential results in removal of stains in combination with detergent .[49] Amylase enzyme used in different industries i.e., Starch, Detergent, Fuel and Alcohol Production, Food, Textile, Paper etc. [50]

PECTINASE

Pectinases are a group of enzymes that degrade pectic substance and are classified according to their mechanism of action. Methoxy groups, for instance, are removed by methylesterases from strongly or partly esterified galacturonan. Exopoly-glacturonases and endopolygalacturonases are two types of polygalacturonases that catalyse the release of galacturonic or digalacturunic acid residues from the hydrolysis of glycosidic bonds in a random manner. [51-52] According to their mode of action and substrate, pectinolytic enzymes, also known as pectinases, are also divided into three groups: polygalacturonases, which are further divided into endo- and exo-polygalacturonases; lyases, which are further divided into pectatelyases or pectin lyases; and pectin methylesterases. Numerous studies on pectinases in nematodes that live in soil have been conducted. With the use of a homogenate of D. dipsaci, Tracey acquired a little degradation of pectic acid found that homogenates of Pratylenchus penetrans and Heterodera trifolii reduced the viscosity of pectin solution. Myers was able to produce pectinase activity utilising pectin substrate from Radopholus similis homogenates as well as larvae of three different Meloidogyne species, but not from Aphelenchoides sacchari, Ditylenchus triformis, or

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Panagrellus redivivus homogenates . In Aphelenchus avenae extracts, Barker discovered a poly-galacturonase that was more active towards Na polypectate than pectin solution and had an ideal pH of about 5.0. According to Goffart & Heiling, pectinases were secreted into solution by Ditylenchus destructor and Meloidogyne hapla ,[53] but Myuge found no pectinase activity in potato tubers infected with D. destructor. Pectinases play a crucial role to reduce the viscosity, increase the yield and clarification of juice by liquefaction of pulps, remove off the peels [55-58] and in maceration of vegetables to produce various products like pastes and purees.[59-61] Along with other cell wall-degrading enzymes like cellulases and hemicellulases, pectinolytic enzymes have also been used in this way.[62] When a combination of two commercial enzymes, pectinase and hemicellulase, was utilized at the extraction temperature of 40 C compared to the control, an improvement in pineapple juice recovery of around 25% was attained. [61] Pectinolytic enzymes enhance the extraction process, maximize juice output, make filtering easier, and improve flavor and color in the wine-making process. Enzymatically treated wines showed more stability with reduced filtration time in comparison to control wines.[63] Before adding inoculum, pectinolytic enzymes were used to macerated grapes, which enhanced the qualities of the wine. [64-65]Additionally, pectinases are employed in biorefineries to hydrolyze the pectin found in pectin-rich agricultural and industrial waste.[66] These wastes are turned into simple sugars so they can be utilized as fermentable sugars or turned into bioethanol.[67-68] Olive, sunflower, coconut, palm, or canola vegetable oils are extracted using hexane, an organic solvent that may be carcinogenic carcinogen. [55] The traditional scouring method, which uses harsh chemicals, is gradually being replaced with an environmentally benign one involving enzymes. Using particular enzymes, bio-scouring is an environmentally benign technique for removing non-cellulosic contaminants from the fibre.[65] By dissolving the pectin found in the cell walls of tea leaves, pectinase treatment speeds up the fermentation of tea. It also eliminates the pectins ability to cause froth to develop in instant tea granules. A distinctive scent also develops as a result of the fermentation-induced change in tea's hue.[69] Pectinases are used in the manufacturing of bovine feed to lower feed viscosity, boost nutrient absorption by ruminants, and release nutrients by enzymatic activity, which also decreases the amount of faeces.[70] Pectinases alone or in conjunction with other enzymes produced by the same or other microorganisms have effectively been utilized for bio bleaching of kraft pulps made from mixed hardwood and bamboo.[71] Enzymatic deinking modifies the bonds close to the ink particle and removes the ink from the surface of the fiber. The discharged ink is then washed off or floated away. It is advised to combine various pectinases with other enzymes like cellulases and hemicelullases in order to maximize the degradation of the pectin in various raw materials, such as citrus juice processing. Multiple enzymes can degrade different parts of the polymer.[72-73] According to studies, microbial pectinase accounts for 25% of all food and industrial enzyme sales worldwide, and its market is continually growing.[72]

LIGNIN DIGESTING ENZYMES

The lignin-degrading enzymes are members of the multi-copper oxidase (AA1) family, which is categorized as an auxiliary activity (AA) enzyme in the CAZy database (Park & Kong, 2018). Generally speaking, lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes are the two primary categories of enzymes involved in the degradation of lignin. LDA enzymes are required to complete the degradation process because they are unable to breakdown lignin on their own.[75] The parasitic nematodes Bursaphelenchus xylophilus (pine wood nematode), Globodera pallida (potato cyst nematode), Heterodera glycines (soybean cyst nematode), and various Meloidogyne species (root-knot nematodes) are among the many plant pathogens that cause significant crop damage and agricultural losses that can reach up to $157 billion annually .[76] The lignin-degrading enzymes are members of the multi-copper oxidase (AA1) family and are categorised as auxiliary activity (AA) in the CAZy database. It's interesting to note that two nematodes, the pine wood and potato cyst nematodes, both produced the lignin-degrading enzymes laccase (Bux.s00116.660 and GPLIN_001134600) and laccase-like (GPLIN_001134500). Lignin peroxidase's structural and functional characteristics have been investigated.[77] LiP is a crucial enzyme for biotechnology with several potential uses in the delignification of lignocellulosic materials, which are seen as an alternative to the depleting oil reserves. In the conversion of coal to low molecular mass fractions)[74], which could be used as a feed stock for the production of commodity chemicals. In biopulping and bio bleaching.[48] In the paper industry, the elimination of organic contaminants that are resistant to removal, and the enzymatic polymerization in the polymer industry. Laccases have emerged as the most promising enzymes for industrial applications during the past 20 years [61] having applications in food, pulp and paper, textile, cosmetic industries and in synthetic organic chemistry.[78] Lignocellulytic enzymes are extremely beneficial for industry, and fungal cellulases are finding new uses in a variety of fields, including the production of fruit juice, ruminant nutrition to increase digestibility, and paper de-inking .[79] Enzymes that break down lignin have a lot of promise in biotechnological and industrial applications. contrasted to the thermal and chemical pretreatment processes for the manufacture of biofuel, an ecologically beneficial option.[80-81] Delignification of lignocellulose, Bio pulping and Bio bleaching, Textile dye transformation.[79] Decolorization of distillery effluent and waste effluent treatment.

Hemicellulase

Hemicelluloses are cellulose-binding polysaccharides that cooperate with cellulose to create a solid but adaptable network. More specifically, they are described as carbohydrate polymers mostly composed of (1,4)- and (1,3) glycosidic linkages and composed of either xylose, glucose, mannose, or mannose and glucose. [82] The second most prevalent polysaccharide in nature, xylan, is a key component of hemicellulose. According to the plant species, xylan is made up of (1,4)- β-linked xylopyranose units and can have a variety of substituents and varied structures .[83] Other components of hemicellulose, such as lichenan, contain both (1,3)-β and (1,4)-β linkages.[84] Nematode GHF5 endoglucanases can show activity against hemicelluloses, but the activity seems limited to (1,4)-β linked polysaccharides.[85] The GHF45 endoglucanases of B. xylophilus are also active against glucomannan.[86] Limited activity of some endoglucanases against lichenan has also been observed.[85] Endoglucanases from Heterodera glycines have shown that some endoglucanases can hydrolyze xylan . Furthermore, unique endoxylanase enzymes have been discovered in just a few nematodes, including Meloidogyne species and Radopholus similis. These xylanases have a domain structure resembling that of endoglucanases, consisting of a signal peptide, a catalytic domain, and occasionally a C-terminal CBM. They are members of the glycosyl hydrolase family located between GHF5 and GHF30.[87] A crucial part in the hydrolysis of lignocellulosic substrates is played by hemicellulases. Heteropolysaccharides called hemicelluloses are made up of several hexoses and pentose[82-84], and the efficient degradation of the polymer requires the synergistic action of many hemicellulolytic enzymes such as xylanases, mannanases, arabinofuranosidases, glucuronidases, xylosidases, and hemicellulolytic esterases (Shallom & Shoham, 2003). Cello-oligosaccharides are produced from beta-glucan of barley using hemicellulose. Hemicellulases are primarily employed in the synthesis of prebiotic oligosaccharides and in the banking sector (Danalache et al., 2018; Toushik et al., 2017).[89-90] Hemicellulolytic enzymes can be utilised as macerating enzymes in conjunction with cellulases and pectinases, depending on the raw material and processing method. The enzyme hemicellulosease breaks down hemicellulose. Despite the diversity of microbial enzyme systems for its biodegradation and the complexity and resistance of plant cell walls, enormous strides have been made over the past 50 years in the development of effective enzyme technologies to transform plant materials into affordable fermentable sugars and to enhance industrial processes and products. Textile, animal feed, bakeries, breweries, detergent, pulp and paper, and biofuel are among the industries that gain from research and development on cellulases and hemicellulases. The need for particular, highly active, and stable enzymes is expanding quickly in the industrial sector. According to estimates, the global market for industrial enzymes was around $4.2 billion in 2014 and is projected to expand at a compound annual growth rate of over 7% between 2015 and 2020 to reach almost $6.2 billion. The major categories of hydrolases, cellulases and hemicellulases, make for around 75% of commercial enzymes.

CONCLUSION

Although the usage of enzymes derived from nematodes is still in its infancy, there is a lot of promise for these enzymes to be employed in a wide range of applications. It is also a promising field of study with the potential to help a wide range of businesses and applications. They are a sustainable and ecologically friendly alternative to conventional insecticides and other chemicals. The enzyme chitinase can be used to control nematodes that harm crops, as well as other specialized uses and advantages of nematode-derived enzymes. Cellulase may be used to break down cellulose, a significant component of plant cell walls, which can assist to enhance the drainage and aeration of soil, making it more conducive for plant development. Chitinase breaks down the chitin that makes up the nematode's exoskeleton, killing the creature. Lipids, which make up a significant portion of plant cell membranes, may be broken down by the enzyme lipase. Increased growth may result from better plant nutrient absorption thanks to this. The nitrogenous substance urea, which is frequently present in dirty water, may be broken down by the enzyme ureolytic, helping to enhance the water's quality and make it safe for ingestion. Proteins, a key component of cancer cells, may be broken down by protease, helping to destroy cancer cells and reduce tumor size. Future applications for these enzymes are likely to be much more creative and advantageous as our knowledge of them expands. Numerous nematode species have not yet had their enzyme compositions examined. We can increase the breadth of possible uses for nematode-derived enzymes by discovering novel enzymes from these species. Characterizing novel enzymes' characteristics, such as substrate selectivity, pH range, and temperature stability, is crucial after they have been discovered. We can better understand how enzymes function and how they may be used by using this information.

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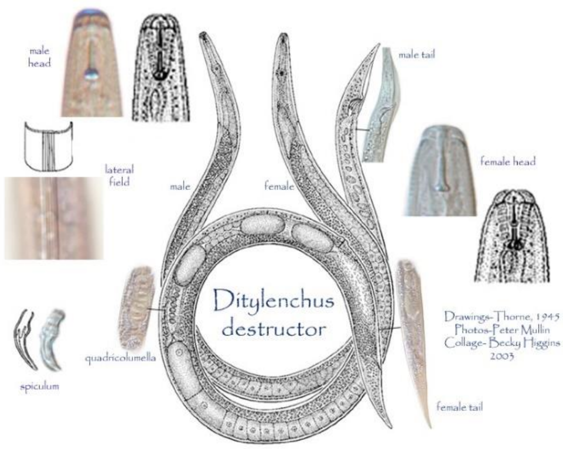

Fig. 1: Ditylenchus destructor
Courtsey:nematode.unl.edu

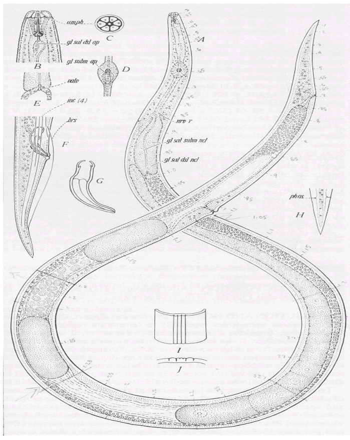

Fig. 2: Ditylenchus dipsaci
Courtsey:nemaplex.ucdavis.edu

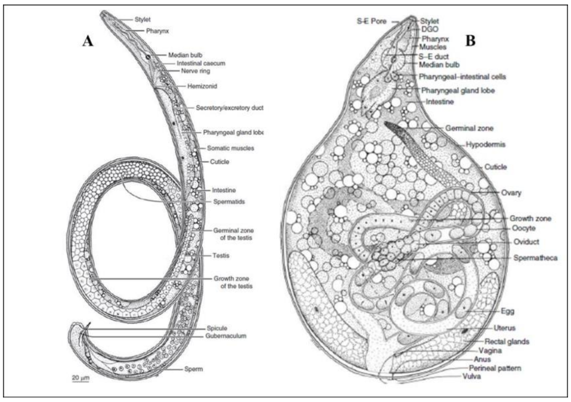

Fig. 3: Meloidogyne incognita
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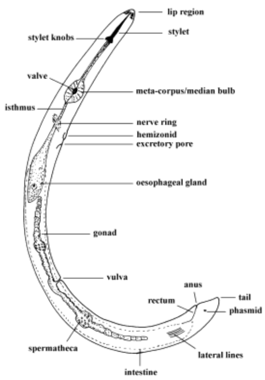

Fig. 4: Heterodera trifolii
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