Predatory nematodes as bio-control agent against plant-parasitic nematode -A review

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ABSTRACT
Nematodes are highly diversified and most abundant metazoans present in soil. Phytophagous nematodes attack plants cause enormous losses. The predaceous nematodes play a significant role in regulating the population of plant-parasitic nematodes. Based on type of feeding apparatus and mode of feeding, predatory nematode are classified into different taxonomic categories. This review has made an attempt to evaluate the bio-efficacy of predaceous nematodes and their future prospects.

Key words: Biological control, Feeding apparatus, Mass culture, Mode of feeding, Predatory nematode.

INTRODUCTION
Nematodes are the most abundant and diverse metazoans present in all types of soils. Based on feeding types, they are microphagous, saprophagous, phytophagous and predaceous. All these types of nematodes occur in soil concomitantly. Phytophagous nematodes attack plants and feed mainly on roots and also above ground parts. Depending on the population level of the nematode the plants show poor growth and disease symptoms resulting into significant crop losses. The rate of reproduction and natural deaths are important factors in regulating population level, but the parasites/pests/predators and other antagonistic animals also play a very significant part in this act. The biological control of plant-parasitic nematodes is thus a means of promoting organisms that are harmless to plant host on one hand and on the other they can attack and kill the harmful plant pests. The predatory nematodes therefore become our natural choice and allies in combating plant nematode infestation. They feed on soil microorganisms as well as plant parasitic nematodes. They have a dual function: they reduce population of plant parasitic nematodes in all types of soil and also release nutrients in plant available forms, which enable plants to better withstand nematode burden on the roots. As a biocontrol agent they offer an ecologically safe alternative to chemical nematicides. The article summarizes the progress of studies about predatory nematodes so far and also future prospects as bio-control agent.

The predatory nematodes belong to four major orders, Mononchida, Diplogasterida, Dorylaimida and Aphelenchida. They have different types of feeding apparatus and modes of prey searching, catching and feeding mechanisms and prey preferences. Species belonging to Dorylaimoidea, Nygolaimoidea, Aphelenchida, Diplogasterida are commonly known as dorylaim, nygolaim, aphelench, diplogasterid predators. The nematodes belonging to Monohysterida, Bathydonotina and Actinolaimoidea are also predaceous as is evident from the structure of their feeding apparatus.

Bastian, (1865) proposed the genus Mononchus and described the species M. cristatus, M. macrostoma, M. papillatus, M. truncatus and M. tunbridgensis. Cobb (1917) first suggested the use of predatory nematode, Mononchus spp. for Tylenchulus semipenetrans biocontrol. Steiner and Heinly (1922) suggested the use of Clarkus papillatus in controlling Heterodera radicicola in sugar-beet fields. Cassidy (1931) concluded that under suitable conditions Leptonchus brachylaimus might partially control populations of pest nematodes. Thorne and Swanger (1936) and Linford and Oliviera (1937) reported several species of Aporcelaimus, Nygolaimus, Sectonema, Labronema, Dorylaimus, Dorylaimoidea and Actinolaimus as predaceous. Yeates (1969) first evaluated the predatory ability of Diplenteron colobocercus (=Mononchoides potothuka) on plant-parasitic nematodes. Investigations were made on their life cycle predatory behavior, ultrastructure and function of feeding apparatus, predation abilities, effect of mechanical stimulation and crowding on predation, factors influencing predation, cannibalistic tendency and intra-specific interactions. Andrässy (1993) contributed significantly to the taxonomy of the genus Mononchus. Species of Seinura (Fuchs, 1931) have been recorded from the pine trees infested with pine wilt nematode, Bursaphelenchus xylophilus from in USA and China (Huang and Ye, 2007). Jana et al. (2010) and Choudhary and Jairajpuri (2011) described Mylonchulus signaturellus and Iotonchus parabasidontus respectively from India. Mahamood (2014) reported Acrostichus
Feeding apparatus of predatory nematodes: The feeding apparatus of diplogasterid predators is of cutting and sucking type. Though, they possess a comparatively small buccal cavity but it is well armed with teeth of different sizes which are located at different positions. The mouth of these predators is circular with 6 pairs of 'rugue' and 10 labial papillae; lips are inconspicuous and fused. The cheilorhabdions, which are anterior in position, form cheilostome of the stoma / buccal cavity, which are undivided and appear to protrude outside the lip region. The dorsal meta-rhabdions are heavily sclerotized and provided with a claw-like tooth, having its apex directed anteriorly. This is called dorsal tooth which is movable and hollow from inside. This is the main killing weapon and also responsible for the ejection of secretions from the oesophageal glands. The subventral walls opposite to the dorsal tooth bear small, rose-thorn shaped teeth while the narrower hinder part of the buccal cavity bears a small tooth at its base. Some species also possess denticles which help in grinding the food particles. Telorhabdions are weakly sclerotized, situated at the posterior end forming the telostome of the buccal cavity.

The barrel shaped stoma or buccal cavity of mononch predator consisting of two sets of three plates each one dorsal and two sub-ventral. The anterior set is more developed, large and vertical while posterior set is small and oblique. The dorsal plate of the vertical set bears a large sharply pointed tooth while the sub ventral wall possess similar or smaller tooth, teeth or denticles, variable in number and arrangement. The buccal armature is responsible for catching, puncturing, cutting into pieces or engulfing the prey as a whole. The food may be the intact prey or its pieces, internal body organs or the body fluid to be sucked. The catching cephalic muscles are of two types: labial muscles are a set of six muscles attached posterior to the pharynx. Towards the anterior region these are bifurcated and each band is connected to a different lip. The outer set is of stomatal muscles which are attached to the vertical walls posterior to the denticles. Two of these muscles are subdorsal, two subventral and four sublateral. The buccal cavity with its associated muscles forms the trophico sensory organ of the mononch predator.

The dorylaim predators have piercing and sucking type of feeding apparatus. With the help of their dagger-shaped odontostyle, they puncture the body wall of the prey and suck its body contents. The feeding apparatus, consisting of a vestibule, guiding ring, guiding sheath, odontostyle and odontophore. The odontostyle lies at the base of the guiding apparatus overlying the odontophore. The odontophore is in continuity with the oesophageal lumen and is usually surrounded by an ellipsoidal swelling. The odontostyle is axial in position and has a dorsal aperture and groove. The prey contents (semi-solid, particulate) are passing through the odontostyle.

The species of Nygloaimoidea, viz. Aquatides and Sectonema, having the large, slender non-axial, protrusible tooth /odontostyle, called mural tooth with which they pierce or slit their prey. The prey contents (semi-solid, particulate) pass through the odontostyle while it is sucked up the combined action of the guiding sheath (pharyngeal cavity) and the basal part of the stoma (telostome or odontophore).

The actinolaim predator can feed by cutting the body of the prey. The vestibule is reinforced with plate-like or ribbed basket-like structures which may frequently be accompanied by large onchia with or without denticles. This additional armature may be helpful in killing the prey and cutting opens its body wall. Three sets of specialized muscles- dilator, protractor and retractor muscles are attached to the feeding apparatus and bring about its forward and backward movements during feeding.

The enoplid predators can feed by cutting the body of the prey. Ironus spp. have three sharply pointed teeth which act as ripping organ for tearing open the tissues of the prey. The large cylindrical pharyngeal cavity acts as sucking organ. Well developed muscles are associated with the feeding apparatus.

Feeding mechanism: Attraction, aggregation, prey catching and feeding: Diplogasterid predators are able to perceive prey attractants besides chance meeting. Probing with head (with the help of papillae and amphid) and pharyngeal pulsations helps the predators identify their prey, locate suitable spot on the prey initiating an attack with a convenient posture at right angles. Probing by diplogasterid is quite aggressive, rapid and consists of side-to-side rubbing for a varying duration and in different times. The prey catching and attack is instant and only one or two attacks are enough to puncture the cuticle of prey. Mononchoids aquaticus attacks its prey by moving the lips backward resulting in the mouth opening wide and dorsal tooth coming in close contact with the prey. M. dentatus initiates attack by widely opening its vestibule with the help of labial muscles and exposing the dorsal tooth and denticles to bite the prey. The cuticle of the prey is ripped upon or punctured.
Extracorporeal digestion takes place through pharyngeal gland secretion. Complex food globules are broken down into small particles that are ingested through the feeding apparatus lumen to the intestine. The ingestion of prey contents is intermittent with brief sucking periods. Feeding in predators is completed soon after the prey is completely consumed. They also feed in groups on a single prey at feeding sites. The duration of aggregation at feeding sites and the actual feeding time may vary from predator to predator and depends on the type of prey, texture and contour of prey cuticle, composition, concentration, quality and quantity of the prey contents. Mononchoides longicaudatus and M. fortidens respond positively towards live and excised bacteriophagous nematodes (Acrobeloides sp., Cephalobus sp., Panagrellus redivivus), ectoparasitic nematodes (Trichodorus sp., Xiphinema americanum, Longidorus sp., Tylenchorhynchus mashhoodi, Paralongidionis sp., Hirschmanniella oryzae, Hemicriconemoides mangiferae, Hoplolaimus indicus, Helicotylenchus indicus), endoparasitic nematodes (second stage juveniles of Meloidogyne incognita and Anguina tritici) as well as bacteria. Group feeding allows predators to finish their prey rather quickly and then continue hunting. All diplogasterid predators viz., Mononchoides longicaudatus, M. fortidens, Butlerius degrissei and Diplenteron colobocercus get hold of their prey with the help of high suction force created by their oesophagus. D. colobocercus, Butlerius sp., M. longicaudatus and M. fortidens feed by puncturing the cuticle and then sucking the contents of the body of prey. However, M. fortidens devour intact first and second stage juveniles of small-sized prey nematodes, Acrobeloides sp., and Cephalobus sp., and also cut larger prey into pieces for feeding purposes.

The attraction and aggregation has been observed in dorylaim predators viz., Labronema vulvapapillatum and Dorylaimus stagnalis. Though, this suggests some kind of chemo attraction but it appears mild and insignificant as only a few predators aggregate around a prey requiring more time to reach the feeding sites. The dorylaim, nygolaim and aphelenchid predators feed by puncturing the cuticle of the prey and then sucking its body contents, afterpenetrating the body of prey the predators move their feeding apparatus sideways. During these movements each odontostyle protrusion is accompanied by a suction which is caused by the contraction of oesophageal muscles. Whenever, the radial muscles within the oesophageal bulb contract, their radial oesophageal lumen is dilated and the bulb is stretched. Upon relaxation and simultaneous shortening of bulb, the oesophageal lumen narrows from front to its back. This allows the sucking in of the body contents and forcing them into the intestine through the oesophago-intestinal junction. The depletion of the prey’s contents at the feeding spot is usually followed by searching movements of the feeding apparatus and finally by the separation of the lips from the prey and subsequent retraction of the stylet/tooth. The same prey is then attacked at some other place and feeding starts all over again. When in contact with their own eggs they rub them with their lips several times but do not feed upon them. Feeding in predators is completed soon after the sucking in the whole body contents of prey except the cuticle.

In case of Seinura spp., once the body of the prey is punctured, the secretions of the pharyngeal glands are poured into the prey with the action of the powerful median pharyngeal bulb. The prey is paralyzed and its partially digested body contents sucked in with the aid of the median pharyngeal bulb creating a suction force.
The feeding mechanism was studied in mononchids, viz. *Prionchulus punctatus*, *Mononchus aquaticus* and *Mylonchulus dentatus* (Grootaert and Wyss, 1979). The food is located by chance encounters and lip contact with prey and this initiates feeding. After encounter, probing follows which consists of rapid side to side movement of lips over the prey cuticle. Once the required posture is attained, the labial muscles contract and pull the lips outwards and backwards, so that the mouth and the vestibule are wide open. The contraction of stomatal muscles helps in widening the stoma and the movement of teeth/tooth. The functioning of tooth is supplemented by a strong suction force generated by the contraction of oesophageal muscles. They do not predigest food since they can swallow a prey whole or ingest its pieces through the wide oral aperture. The swallowing of whole prey is aided by the contraction of oesophageal muscles which pull the prey into the oesophageal lumen and the posterior oblique plates assuming vertical position. The stomatal muscles along with the suction probably provide a good hold on the struggling prey. Further, after the feeding has been accomplished these muscles restore the head region to its original position thereby acting as retractors. The act of devouring an entire prey may lead the *Mononchus* spp. to move randomly once again in search of yet another prey but sometimes they also become inactive for short durations. Feeding by predators is completed soon after the prey is completely consumed or devouring an intact prey.

**Biology and ecology:** The duration of life cycle varies from species to species. Generally diplogasterids complete their life cycle in one week. *M. fortidens* takes 4-7 days; *M. gaugleri* takes 6.4 days while *O. longicaudata* takes 13-14 days to complete each life cycle. Dorylaimids generally take 3-6 months to complete one life cycle, however *Labronema vulvapapillatum* completes a life cycle in 36 days at 25°C. *Seinura* spp. completes their life cycle in 3-6 days. *Mononchus aquaticus* takes 15 days while *Prionchulus punctatus* takes 45 days at 25°C to complete one generation. Mononchids were found prevalent at pH range of 5.78-6.58, soil temperature between 20.6-23.0°C, moisture levels between 17.71-24.53% and organic carbon contents between 3.06-4.28% around the plant roots (Sharma and Gupta, 2015).

**Factors influencing predation:** Different biotic and abiotic factors increase or decrease the rate of predation.

**Prey activity:** Predation depends on the type of prey. Predation by *M. aquaticus* inversely proportional to the activity of the prey. The most active prey, *Prismatolaimus* sp., was killed least, while the least active *Cephalobus* sp., was devoured most.

Sex and age of predators: Males of *D. colobocercus* prey more than their females. Adult and fourth stage juveniles being stronger, kill a larger number of prey than their young stages.

**Prey number:** The prey density affects the rate of predation by increasing chances of encounters. Rate of predation by *D. colobocercus*, *A. thornei*, *M. longicaudatus* and *M. fortidens* is proportional to prey density.

**Temperature:** There are usually two types of effects that can be produced by temperature: one is on the sensory behavioral responses mediated through the sensory receptors and the other is thermodynamic effect resulting from changes in the rate of metabolic processes of the nematodes. Temperature influences the activity of predators and prey and cause decline in the rate of predation by *M. aquaticus*. The optimum temperature for diplogasterid predators was found to be between 25-30°C. *D. stagnalis* preyed maximum at temperatures ranging between 25-30°C (Shafqat et al. 1987).

**Starvation:** The short-term deprivation of food enhances predation by *P. punctatus*, but declines when the predators are starved for a long duration.

**Prey preference:** The prey selection or preference may differ from species to species of predators. The selection of prey depends on the activity, size and the behaviour of prey nematodes. Diplogasterids are prey selective. Thorne (1932) found remnants in *Iotonchus acutus* of those prey (*Rotylenchus robustus*, *Trichodorus sparsus*) which were not killed by *Butlerius* sp., *Butlerius* sp. preferred soil stages of endoparasitic nematodes in place of ectoparasitic ones (Grootaert et al. 1977). *Rotylenchus robustus*, *Tylenchus macrurus*, *Meloidogyne naasi*, *Pratylenchus sp., Rhabditis sp., Plectus sp.* and *Mesodorylaimus sp.* were attacked rarely but served as food for *Butlerius* sp., when artificially wounded. *Mononchoides longicaudatus* and *M. fortidens*, *M. gaugleri* preferred juveniles of endoparasitic over ectoparasitic nematodes. *M. aquaticus* preferred less active nematodes like *Cephalobus sp.*, *Aglenchus parvus* as compared to more active *Prismatolaimus* sp. *M. longicaudatus* and *M. fortidens* prefer *Acrrobelloides sp.*, *Cephalobus sp.*, *P. redivivus*, *Hirschmanniella oryzae* and the second stage juveniles of *Meloidogyne incognita* and *A. tritici*, but did not feed upon *Hoplolaimus indicus* and *Hemicriconemoidea mangiferae*. Other prey nematodes, viz., *Rhabditis sp.*, *Longidorus sp.*, *Xiphinema americanum*, *Tylencythonchus mshhoohdi* and *Helicotylenchus indicus* were moderately preferred. Similarly feeding by *Mylonchulus signatus* was more on *Tylencythonchus semipenetrans* or *Meloidogyne javanica* juveniles than on *Helicotylenchus multicinctus* or *Longidorus africanus*. High degree of prey specificity was reported in *O. longicaudata*, *A. pacifica*, *Butlerius*, *Mononchoides* (Chitambar and Noffsinger, 1989; Bilgrami et al. 2005).
Mononchs prefer Meloidogyne spp., Pratylenchus spp., Paratylenchus spp., Meloidodera spp., Tylenchorhynchus spp. etc.

The dorylaim predator, Labronema vulvapapillatum preferred Aphelechnus avenae, Panagrellus redivivus and Anguina tritici in place of Pratylenchus penetrans and Xiphinema index. Eudorylaimus obtusicaudatus feeds on eggs of Heteroder a schachtii and Thormia spp. and decreases the population of citrus nematode.

Prey resistance: The ability of different prey nematodes to defend themselves from predators may vary from species to species and from individual to individual and determines the degree of their resistance/susceptibility to predation. The resistance is of varied type and it may be physical, chemical or behavioral. Physical resistance is due to physical characteristics such as, thick cuticle, body annulations, double cuticle and gelatinous matrix. The chemical resistance is because of toxic / unfavourable / repellent secretions while behavioural characteristic represents certain mechanisms of the prey nematodes to resist or to avoid predation by active body undulations, ability to retreat instantly when attacked, vigorous escape response and the inability of predators to attack and injure them (inert mechanisms). A wound may result in loss of hydrostatic pressure of body aff ecting locomotion and thereby making prey more vulnerable to predation. Wounding is the only intermediate factor between the mortality and survivality of prey organisms and hence could be taken into account for determining the degree of resistance of prey and susceptibility to predation. Hoplolaimus tylenchiformis, Belonolaimus longicaudatus, Hemicydiophora similis, Dolichodorus heteroccephalus, Cricnomoides spp., Scutellonema spp., and Helicotylenchus spp. are resistant prey species. Tylenchorhynchus sp., Tylenchus macruris, Meloidogyne naasi, Merlinus sp. Pratylenchus sp. (adults), Trichodorus sparsus and Hemicydiophora sp. were totally resistant to predation by Butlerius sp. The high degree of susceptibility of Cephalobus sp., Acrobelo ides sp., P. redivivus along with the second stage juveniles of M. incognita and A. tritici to predation by M. longicaudatus and M. fortidens happened to be due to their small body size, slow rate of movement and lack of protective cuticle adaptations.

Mononchus spp. may overcome resistance of prey of different kinds to a larger extent as these predators often do not cut the cuticle of prey but engulf them whole. The ectoparasitic nematodes are, therefore, more vulnerable to predation by Mononchus spp. This is evident as many species of Mononchus have been found containing species of Hoplolaimus, Helicotylenchus, Xiphinema, Hemicricnomoides, Rhabditis, etc., besides other Tylenchus spp., dorylaims, rhabditids and Monochus spp. entire in their intestine. The escape response may be more successful against a comparatively slow predator like L. vulvapapillatum, D. stagnalis, A. thornei and probably against all dorylaim and nygolaim predators which require several stylet thrusts to achieve penetration. Aphelenchid predators, Seinura spp. may overcome escape responses of their prey with the help of their toxic secretions which paralyze the prey quickly.

Cannibalism: The tendency of feeding on their own members is mostly observed in Mononchus spp. The cannibalistic tendency may vary from predator to predator as species of Mononchus spp. and Sporochalus sp. killed their own species more than the other mononchus (predators). Cannibalism appears to be more of natural phenomenon rather than occurring only due to non-availability of prey as in most instances the Mononchus sp. (prey) occurred together with other types of prey in the intestine of Mononchus sp. (predators). The ability of predators (dorylaims, nygolaims, diplogasterids) to recognize their own chemical secretion and to attract their own members possibly induces cannibalism. In M. aquaticus besides adults, the fourth and third stage juveniles also show cannibalistic behaviour when placed with their own juvenile stages in absence of prey nematodes. All species except P. muscorum, Coomansus indicus, Itonchus basidontus, I. jairi, I. baqrii, I. shafi, I. antedontus, I. longicaudatus, I. prabhoi and Myconchus spp. show cannibalistic tendency. Seinura spp. also cannibalistic when prey are in short supply.

Culture of predatory nematodes: Besides prey nematode, the predatory nematodes feed on other soil microorganisms. Diplogasterids can be reared on either prey nematodes or various bacteria both by in vivo or in vitro methods since they are facultative and biphasic. Diplonteron colobocercus, B. degrissei, M. fortidens, M. longicaudatus have been maintained on nematodes like Caenorhabditis, Rhabditis, Panagrellus, Cephalobus, bacteria (Escherichia coli) or on a combination of prey nematodes. Yeates (1969) cultured in vitro diplogastrid predator D. colobocercus on agar plates using a bacterium, Bacillus cereus var mycoides. Mononchoiides fortidens can be cultured on Rhabditis sp. on agar supplemented with skim milk powder. Nelmes (1974) reared Prionchulus punctatus on A.avenae and P. redivivus in soil extract agar medium at pH 6.8. M. aquaticus and P. punctatus can be multiplied by using P. redivivus as prey. Koerneria sudhausi can be cultured on bacteria that developed on a nutrient media (Meira et al. 2008).

Potentiality of biocontrol: Single Mononchus papillatus killed 1332 Heteroder a radicicola juveniles in its lifespan. Predatory activity of Myconchus sigmaturas resulting in low population of Tylenchulus semipenetrans (Cohn and Mordechai, 1974). Azmi (1983) found an increase in number of Itonchus monhystera and reduction of Helicotylenchus dihystera. Small and Grootaert (1983) reported significant reduction in the population densities of Globodera rostochiensis and Meloidogyne incognita in presence of
Prionchulus puncatus. Seinura paynei were found feeding on free-living nematodes, e.g. Acrobeloides sp., Barsilla labiata and Aphelenchoides richardsoni (Grewal et al. 1991). Fauzia et al. (1998) demonstrated the ability of M.longicaudatus to reduce root knot nematode population. Iotonchus tenuicaudatus reduces the population of Tylenchulus semipenetrans and H.dihystera in mandarin orange orchard (Rama and Dasgupta, 1998). Khan and Kim (2005) reported that M.fortidens reduced M.arenaria population in tomato. Bilgrami et al. (2008) have demonstrated that M.gangleri reduced the population of naturally occurring plant-parasitic nematodes in a turf grass field in USA.

Conservation: Population of predatory nematodes, viz., Dorylaimus spp., Discolaimus spp. and Mononchus spp. increases when green manure is used in soil. Their population densities can be raised by adding organic amendments like leaves, litter that support bacterial and fungal growth under natural conditions. Dorylaimid and mononchids are more abundant in the soil with improved texture as they are larger in body size. Soil amendments with neem products such as leaf powder, oilseed cake, sawdust help in maintaining and conserving predatory nematode population, they are simple and cost effective. Winter mulching also improve predatory nematode conservation and stabilizes the population of Iotonchus tenuicaudatus that feed on Tylenchulus semipenetrans and Helicotylenchus dihystera in orange orchards (Rama and Dasgupta, 1998).

Future prospects of predatory nematodes: The knowledge of biology, food and feeding preferences, predator-prey relationship, range of prey together with other ecological parameters has great significance in formulating biological control programmes and in proper utilization of different kinds of predators. The degree of attraction, duration of post-feeding aggregation, duration of feeding sites and actual feeding time may vary from individual to individual and species to species depending upon the type of prey, composition, concentration, quality and quantity of prey secretions/ attractants. These may be important determinant factors. Because of their long life-cycle, low rate of fecundity, susceptibility to changing environmental conditions, cannibalism, difficulty of mass production, Mononchus spp. are not considered as efficient biocontrol agents. Dorylaim, nygolaim and aphelenchid predators can switch to feeding on bacteria, algae and fungi. Their widespread and abundant presence reflects the possibility of controlling nematode populations. Their population can also be manipulated in the field by adding organic amendments. Among the different types of predators, diplogasterids are the most suitable, as they possess significant potential to reduce population of plant parasitic nematodes. The positive attraction of diplogasterids towards prey, instantaneous prey catching and attack, faster rate of feeding, high rate of predation, high degree of prey specificity, bacteriophagous nature, more tolerant to adverse conditions as evident from the formation of dauer juvenile, short life cycles, easy culture conditions, wide range of prey and probably higher longevity, are some of the characteristics which suggest these predators as efficient agents of biological control of plant-parasitic nematodes. Impact of predatory nematode can be increased during the peak season of hatching of endoparasitic nematodes as the predation rate is density dependent. The lower and higher temperatures other than the optimum also adversely affect both the attraction and predation. These factors could thus be manipulated to modify the activities of predators to maximum extent. Much research is needed to assess the diversity of predaceous species present in different agro-climatic regions, their biology, ecology, behavior, reproductive potential and longevity and to develop technique for their mass culturing, formulation, time and mode of application before making practical use of these predators.

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