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Review Article

Entomopathogenic nematodes as a lethal bioagent to deter *Pieris brassicae* L. (Lepidoptera: Pieridae) infesting different cole crops: A review

WASIM MUZAMIL DASS[1*], HIDAYATULLAH TAK[1], TARIQUE HASSAN ASKARY[2], AB AHAD BHAT[1], ABID HUSSAIN BHAT[1] and FAYAZ HUSSAIN MIR[1]

[1]*Department of Zoology, University of Kashmir, Srinagar – 190006, Kashmir, India*

[2]*Division of Entomology, Faculty of Horticulture SKAUST-K, Shalimar – 190025, Jammu and Kashmir, India*

**Corresponding author E-mail:* wasimmuzamil913@gmail.com

ABSTRACT: Cole crops, the most important and attractive vegetables, belonging to the family Cruciferae are widely grown all over the world. In India, their annual production has been reported to be about 191.77 million metric tons, so chips into the economy by a huge amount. *Pieris brassicae,* one of the dreaded insect pests incur damage to different vegetables to a tune of 40%. To curb this pest damage various synthetic insecticides are applied, albeit showing promising results yet posing drastic negative imprints on non-target organisms, environment and pest resistance. These drawbacks demand a perfect alternative which seals all the glitches created by the chemical insecticides. For this Infective Juveniles (IJs) of Entomopathogenic Nematodes (EPNs) act as perfect biocontrol agents. So, work done in these lines has been reviewed to facilitate students and researchers for advanced research related to entomopathogenic nematodes and *P. brassicae*.

KEYWORDS: Infective juveniles, *Heterorhabditis*, *Steinernema*, synthetic insecticides

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INTRODUCTION

Cole crops are the vegetable crops which are named because of their inflorescence that is devoid of bracts, having flowers consisting of four sepals, which are free and four alternating free petals, four free long and two short stamens and in them, seeds in the fruit are in rows separated by a thin septum. Cole crops are placed taxonomically in the family Brassicaceae or Cruciferaceae which in Latin means cross because of the structure of flowers with four petals that look like a cross. These cole or cruciferous crops are among the large group of vegetables of temperate climates consisting of very important crops such as cabbage (*Brassicae oleracea* var. *capitata*), cauliflower (*Brassicae oleracea* var. *botrytis*), brussels sprout (*Brassicae oleracea* var. *gemmifera*), broccoli (*Brassicae oleracea* var. *italica*), knoll khol (*Brassicae oleracea* var. *gongylodes*) and kale (*Brassicae oleracea* var. *acephala*). These vegetable crops engender from the Mediterranean era (Nieuwhof, 1969), and then introduced in India when it was ruled by the Mughals (Das 1992; Sood 2007). Vitamin C or ascorbic acid is abundant in these crops and are plentiful source of minerals like magnesium, iron, phosphorous, etc. Cole crops have high nutritional value as these crops are rich in vitamins A and C, iron, magnesium, calcium and dietary fibre (Guerena, 2006). There is a huge quantity of antioxidant sulphoraphanes in broccoli which are anticarcinogenic compounds and these compounds are deemed to nullify the effect of UV rays on the eyes and are very helpful in saving us from macular degeneration (Gao & Talalay, 2004), keep the heart ailments, stroke and high blood pressure at bay (Wu *et al.,* 2004). These crops are grown in the winter season and are likely to grow maximally at 60-65°F. They can tolerate slight frost without having any damage or injury. Some varieties continue to grow at temperatures as low as 41°F however few varieties grow maximally above 78°F (Hodges & Neild, 1992). So these cole crops are tremendously cultivated in tropical and subtropical areas of the earth (Das, 1992). All cultivated varieties of cole crops belong to the species *Brassica oleracea* (Haynes *et al.,* 2009). Every cole crop has cognate genome size (2n=18 chromosomes) as that of wild species of *Brassica* that grow along the Atlantic coasts of France, Britain as well as in the Mediterranean basin (Snogerup, 1980). Regarding the location of incipient introduction into the domestication of wild plants and their further domestication process, a query has erupted among scholars and scientists (Candolle, 1885). Knowing the history and evolution of crops can help to

trace current wild and cultivated diversity that could result in increasing and enhancing utilization and conservation. For this, the general hypothesis is that the fleshy and tender shoots of wild *Brassica* sp. were interested and established in home gardens subsequently leafy kale got domesticated. In European gardens, this crop is currently so widespread that it is even grown by elderly persons. It is generally deemed that the Atlantic forms were referred to as the cultivated form's progenitor (Bailey, 1922). They diverged and were introduced to different regions of the world.

Organic production of cole crops depends upon management techniques that keep the fertility of soil for a long time by optimizing the biological activity of soil. For this to achieve cover cropping, crop rotation and composting is done. A healthy well well-balanced soil besides producing quality crops can also abet the development resistance of plants to diseases and insect pests. For pest control, farmers have to manage insects, diseases and weeds with mechanical, cultural and biological methods. To accomplish the life cycle of cole crops they are in requirement of some nutrients in particular to encourage maximum growth and reproduction. The most important nutrient which cole crops need is nitrogen. Nitrogen in these crops is most important for overall growth and for optimum yield good amounts of it are needed. Potassium requirements for cole crops are also high. Calcium as macronutrient and micronutrients, e.g., manganese, molybdenum, boron and iron are sine quo none for cole crop development. Soils active in organic matter have enough of these nutrients to sustain the growth of these crops maximally.

China is the largest producer of cole crops in the world followed by India. Among cole crops cauliflower and broccoli are maximally produced by China producing almost 40% of the world's production followed by India with an estimated production of 9 million tons accounting for 34%. The USA is ranked third. However, this production is depleted and devastated by various insect pests in large amounts. In India vast portion of vegetables are destroyed by insect pests and about 40% of the production of vegetables is damaged alone by *P. brassicae* (Sood, 2007). On cole crops Pajmon listed 38 insect pest species, among them *P. brassicae* is at the pinnacle regarding destruction (Bhalla & Pawar, 1977), crippling all stages from seedling to flowering (Siraj 1999; Younas *et al.,* 2004).

From Europe *P. brassicae* originated and in the wild 91 species from 12 families of crops get infested by it (Someya *et al.,* 2007). *P. brassicae* by nature is wandering without any permanent identifiable colony and reproduces when they find a suitable condition. With optimum circumstances large number of cabbage butterflies explode in strong migration. For this rationale and by encountering huge levels of parasitism number of these insects rejiggers greatly in a given locality from year to year. This pest commonly called large white originated first in Europe and has had a usual presence throughout Europe, North Africa and Asia thereby damaging different crops severely. It was deemed to spread to Australia and New Zealand in 1995, but in December 2014 it was officially declared to be eradicated (Klein, 2016). It was first reported from Delhi in India in February 1996 and its high incidence was very unusual as it resides in mountainous areas (Bhalla *et al.,* 1997). In Kashmir, a mass migration of cabbage butterflies was observed on 28th May 1988 and about 75000 to 80000 butterflies passed in the direction towards the north at an altitude of 3800 to 4000 meters in 1.5 days (Jamdar, 1991).

DEVELOPMENTAL RESPONSE

The life history of *P. brassicae* is dramatically affected by host plants. Survival, sex ratio, oviposition period and rate of development are affected by different hosts (Hasan & Ansari, 2010). *P. brassicae* damages onions, nasturtium, capers, mignonette and hedge garlic in addition to cole crops (Ovsyannikova & Grichanov, 2009). The cosmopolitan pest could infest heavily on about 15 crop plants including cabbage, mustard, cauliflower, radish, turnip, rape etc. (Hwang *et al.,* 2008). The said pest can destroy all stages of the plant including seedling, vegetative and flowering parts. Caterpillars which are young feed heavily on every part of the host plant (Hasan & Ansari, 2011). The caterpillars of *P. brassicae* can feed on every part of cole crops be it a leaf, a fruit, twig or seeds of cabbage, cauliflower and other cole crops. Young larvae of this pest could eat up to 74 to 80 cm^2 of leaf resulting in huge damage to host plants (Younas *et al.,* 2004), thus dwindling its production heavily. This pest could be the rationale for 40% destruction of different crops annually in India (Ali & Rizvi, 2007). Infestation up to an extreme level could completely devastate foliage ultimately resulting in the death of the whole plant (Hasan & Ansari, 2010). The total period for development was highest on yellow sarson 38 to 43 days under laboratory conditions and in the field, it was 36 to 40 days. On cabbage, it was at least 32.35 to 35.95 days and 31.17 to 35.07 days under laboratory and field conditions respectively. Also, its highest mortality per generation was on yellow sarson (0.3565) under laboratory conditions and (0.3645) in the field and the lowest mortality of (0.2555) in laboratory conditions and (0.2486) in the field was on cabbage (Arshad & Rizvi, 2007). Its net reproductive rate reaches its pinnacle on cauliflower which is 27.1 and 2nd highest rate is on cabbage which is 24.89 (Ovsyannikova & Grichanov, 2009). Damage arises economically when leaf area lacerate reaches 3.97 to 10.41% in cabbage at early stages and it is 10.75 to 13.76 in cabbage

Table 1. Distribution of *Pieris brassicae* in different parts of world

Continent	Country	Distribution	Reference
Africa	South Africa	Present (introduced in 1994)	(Claassens, 1995)
	Tunisia	Present	(Higgins, 1983)
	Morocco	Present	(Higgins, 1983)
	Libya	Present	(Larsen & Nakamura, 1983)
	Egypt	Present (few occurrences)	(Larsen & Nakamura, 1983)
	Algeria	Present	(Higgins, 1983)
Asia	Afghanistan	Present	(EPPO, 2022)
	Armenia	Present	(Higgins, 1983)
	Azerbaijan	Present	(Mustafaeva, 1989)
	Bangladesh	Present	(EPPO, 2022)
	Bhutan	Present	(EPPO, 2022)
	China	Absent, Unconfirmed presence record(s)	(EPPO, 2022)
	Georgia	Present	(Higgins, 1983)
	India	Present	(EPPO, 2022)
	Iran	Present	(EPPO, 2022)
	Iraq	Present	(Higgins, 1983)
	Japan	Present, Few occurrences	(Matsuda et al., 1997)
	Hokkaido	Present	(Sato & Ohsaki, 2004)
	Jordan	Present	(Higgins, 1983)
	Kazakhstan	Present	(EPPO, 2022)
	Kyrgyzstan	Present	(EPPO, 2022)
	Lebanon	Present	(Higgins, 1983)
	Myanmar	Present	(EPPO, 2022)
	Nepal	Present	(Thapa, 1987)
	Pakistan	Present	(Shah & Rafi, 2016)
	Syria	Present	(Higgins, 1983)
	Tajikistan	Present	(EPPO, 2022)
	Turkey	Present	(Higgins, 1983)
	Uzbekistan	Present	(EPPO, 2022)
Europe	Austria	Present, Widespread	(Higgins, 1983)
	Belarus	Present, Widespread	(Bunyakin, 1995)
	Belgium	Present	(Higgins, 1983)
	Bulgaria	Present, Widespread	(Higgins, 1983)
	Croatia	Present	(Higgins, 1983)
	Cyprus	Present, Widespread	(Higgins, 1983)
	Czechia	Present, Widespread	(EPPO, 2022)
	Czechoslovakia	Present, Widespread	(Higgins, 1983)
	Denmark	Present	(Higgins, 1983)
	Finland	Present, Few occurrences	(Higgins, 1983)
	France	Present, Widespread	(Higgins, 1983)
	Corsica	Present	(EPPO, 2022)
	Germany	Present, Widespread	(Higgins, 1983)
	Greece	Present	(Higgins, 1983)
	Crete	Present	(Higgins, 1983)
	Hungary	Present, Widespread	(Higgins, 1983)

	Ireland	Present, Widespread	(Higgins, 1983)
	Italy	Present, Widespread	(Higgins, 1983)
	Sicily	Present	(Higgins, 1983)
	Latvia	Present	(Zariņs & Eglite, 1993)
	Lithuania	Present	(Bartninkaite & Tyakoryute, 1994)
	Netherlands	Present	(Higgins, 1983)
	North Macedonia	Present	(EPPO, 2022)
	Norway	Present	(Higgins, 1983)
	Poland	Present	(Higgins, 1983)
	Portugal	Present, Widespread	(EPPO, 2022)
	Azores	Present	(EPPO, 2022)
	Madeira	Present	(EPPO, 2022)
	Russia	Present, Localized	(EPPO, 2022)
	Central Russia	Present, Widespread	(Popova, 1996)
	Eastern Siberia	Present, Localized	(EPPO, 2022)
	Russia (Europe)	Present, Localized	(EPPO, 2022)
	Siberia	Present, Localized	(Higgins, 1983)
	Western Siberia	Present, Localized	(EPPO, 2022)
	Serbia	Present	(EPPO, 2022)
	Serbia and Montenegro	Present	(Higgins, 1983)
	Slovakia	Present	(EPPO, 2022)
	Spain	Present	(Higgins, 1983)
	Balearic Islands	Present	(EPPO, 2022)
	Canary Islands	Present	(EPPO, 2022)
	Sweden	Present, Widespread	(EPPO, 2022)
	Switzerland	Present, Widespread	(Vorbrodt & Müller-Rutz, 1911)
	Ukraine	Present	(Yastrebov, 1991)
	United Kingdom	Present, Widespread	(Thomas, 1989)
	Channel Islands	Present	(Higgins, 1983)
	England	Present, Widespread	(EPPO, 2022)
	Northern Ireland	Present	(Aldwell, 2003)
	Scotland	Present, Widespread	(Thomas, 1989)
Oceania	Newzealand	Eradicated	(EPPO, 2022)
South America	Chile	Present, Localized	(Neira et al., 1989)

at latter stages and in cauliflower it 13.80 to 16.89 at early stages and 7.79 to 9.63 in later stages (Jogar *et al.,* 2005). To avoid this destruction caused by *P. brassicae* different kinds of insecticides viz carbofuran, malathion, monocrotophos, endosulfan, menazon, dimethoate etc. are being used to manage this pest from crop destruction. Against larvae of *P. brassicae* a study was carried out regarding the toxicity of commercial-grade insecticides and it was proved that carbofuran was most toxic at LC 50 and LC 95 levels. There is quite a difference with other insecticides regarding their lethality at LC 50 and LC 95. Regarding toxicity, the order is carbofuran, monocrotophos, fenitrothion, endosulfan, tetrachlorvinphos, menazon, dichlorvos, cytrolane, carbaryl, diazinon and dimethoate in descending order at LC 50 (Deshmukh & Adarsh, 1971). Albeit these synthetic insecticides prove detrimental to *P. brassicae* but side by side they are also detrimental to other organisms which could be beneficial. Application of these synthetic insecticides causes every kind of environmental pollution whether soil, air or water. So to deter the destruction caused by *P. brassicae* to different cole crops and to dwindle the negative impact of these chemical insecticides there is a need for some alternatives that could seal the glitch created by synthetic pesticides. For it, different kinds of biopesticides should be applied. Among biopesticides, EPNs of the two families Steinernematidae and Heterorhabditidae are promising biocontrol agents as they have a wide host range, short life cycle, are easy to mass produce, are cost-effective, eco-friendly and can resist

unfavourable conditions (Askary, 2010). EPNs as the name indicates entomon means insect and pathogenic means they kill insects which damage crops. Using them as an alternative to chemical pesticides could decrease the negative impact of synthetic insecticides and farmers could shift towards them. EPNs are lethal nematodes which act as parasites to kill crop insect pests having high bioefficacy, nonhazardous to non-target organisms including humans and the virtual environment is fully safe by these mini creatures. These tiny creatures harbour mutualistic enterobacteria in their intestine. After making their way into the host insect they ultimately reach hemocoel, where the bacteria get released from them and thereby the production of toxic substances by bacteria, the host insect ultimately gets killed within a short time by septicemia (Woodring & Kaya, 1988).

HISTORY OF ENTOMOPATHOGENIC NEMATODES

Allantonematidae, Mermithidae, Phaenopsitylenchidae, Sphaerularidae, Tetradonematidae, Steinernematidae and Heterorhabditidae are seven nematode families which could control insect pests and act as biological control agents (Kaya & Stock, 1997). However, Steinernematidae and Heterorhabditidae are very beneficial from a biological control point of view and are at the gaze of researchers (Lacey *et al.,* 2001). These nematodes of genus Steinernematids and Heterorhabditids, mutualistically harbor bacteria of genus *Xenorhabdus* and *Photorhabdus* respectively. The complex of nematodes and bacteria is very lethal inactivating the host within 48 hours and having massive reproductive potential (Kaya & Gaugler, 1993). These nematodes infect a large number of hosts but cannot pose any harm to plants, invertebrates and vertebrates including humans (Akhurst, 1990). Regarding biological control EPNs from 2 families viz Steinernematidae and Heterorhabditidae are deployed to control insect pests and are commercially produced to manage insect pests. Heterorhabditidae comprises a single genera *Heterorhabditis* while as Steinernematidae consists of 2 genera *Steinernema* and *Neosteinernema* (Kaya & Gaugler, 1993), which have a mutualistic association with bacteria of genera *Xenorhabdus* and *Photorhabdus* for *Steinernema* and *Heterorhabditis* respectively (Boemare, 2002). Hence in a real sense, it is the complex of nematodes and bacteria that work together as a unit to tackle and curb insect pests including *P. brassicae*.

Aplectana kraussie was the first EPN to be reported by Steiner (1923) and Travassos (1927) named it as *Steinernema kraussie*. However potential for biological control was recognized and assessed by Glasser (1932) when he by the application of *Neoaplectana glaseri* reported the suppression of Japanese beetle. Moreover, the outcome of the application of these minute creatures to 73 plots in New Jersey was 0.2-81% pest management and these nematodes were found in the same soil after 8.5 years of treatment (Glaser & Farrell, 1995).

ECOLOGY AND DISTRIBUTION

Bedding and Akhrust (1975) formulated a baiting technique wherein globally random soil surveys were conducted to find EPNs in tropical, subtropical and temperate climates of different countries. These nematodes were commonly found in every kind of soil whether cultivated or uncultivated and their distribution was to be cosmopolitan (Hominick, 2002). The prevalence of these nematodes are found mostly in Asia and Europe (Table 1). Biologically Steinernematids were heavily diversified than Heterorhabditids. *Steinernema carpocapsae* was distributed widely and has been found in India, America, New Zealand, Australia and Europe. Following in the second position regarding distribution was *S. feltiae* which was found to be distributed from India, New Zealand, Australia and Europe (Poinar, 1990). In temperate regions, *S. carpocapsae* and *S.*

Table 2. Distribution of entomopathogenic nematodes in different countries of world

Entomopathogenic nematode spp.	Countries from where reported	References
Steinernema seemae and *S. masoodi*	India	(Ali *et al.,* 2005)
S. robustispiculum	Vietnam	(Phan *et al.,* 2005)
S. khoisanae	South Africa	(Nguyen *et al.,* 2006a)
S. leizhouense	Southern China	(Nguyen *et al.,* 2006b)
S. ashiuense	Japan	(Phan *et al.,* 2006)
S. sichuanense	China	(Mracek *et al.,* 2006)
S. weiseri	Turkey	(Unlu *et al.,* 2007)
S. costaricense and *S. puntauvense*	Costa Rica	(Uribe-Lorio *et al.,* 2007)
H. safricana	Western Cape Province of South Africa	(Malan *et al.,* 2008)
H. Georgiana	Georgia, USA	(Nguyen *et al.,* 2008)
H. indica	India	(Poinar *et al.,* 1992)
H. amazonensis	Venezuela	(Morales *et al.,* 2016)

feltiae were distributed widely. *H. bacteriophora* was found in the continental Mediterranean climate. Throughout tropics and subtropics *H. indica* was profusely present (Hominick, 2002). *S. anomaly, S. rare, S. kushidai* and *S. scapterisci* were distributed thinly and found only in Russia, Brazil, Japan and Uruguay respectively. So being almost omnipresent, having a wide host range, eco-friendly, etc. they can be harvested, cultured, commercialised and applied in the field against *P. brassicae* and other insect pests which cause immense devastation of cole crops in every part of the world. Apart from these facts they can be applied only once and there is no need to apply them again and again so far as soil application is concerned as they remain in the soil for a long time (Glaser, 1932) provided the moisture in the soil should not diminish below what is necessary for the survival of these wonderful mini creatures. These creatures like other organisms living in terrestrial environments have developed a unique mechanism for survival and resisting extremes of environment like extreme temperature, absence of water, osmotic stress etc. The survival and attacking ability of *S. carpocapsae* is greater at lower temperatures of 5-25°C than at higher temperatures of 35°C whereas the above said qualities in the case of *S. glaseri* were greater at higher temperatures of 15-35°C than at the lower temperature of 5°C (Kung & Gaugler, 1991). In general cooler temperatures have no adverse effect on nematode survival (Kaya, 1990) but the higher temperature could prove detrimental to the nematode survival and pathogenicity (Schmiege, 1963).

The nematodes which are so crucial and could prove fruitful are present almost all over the world. Here is a list of some nematodes present and reported from different parts of the world by different workers.

LIFE CYCLE OF ENTOMOPATHOGENIC NEMATODES

Their life cycle, includes egg, four juvenile stages and adult. The third juvenile stage is a free-living and infective stage called infective juveniles (IJs) that only can infect the host. In the environment or soil the IJs are not feeding because their mouth is sealed and pharynx is collapsed (Kaya & Gaugler, 1993). When these IJs enter the host there occurs a shift from free-living to active parasitic life called reactivation (Hawdon & Schad, 1991). When feeding resumes there are clear changes in morpho+logy as the mouth opens up for feeding, the pharynx straightens up and the pharyngeal bulb becomes visible (Lu *et al.,* 2017). The gut of infective juveniles of both *Heterorhabditis* and *Steinernema* harbour bacteria of the genus *Photorhabdus* and *Xenorhabdus,* respectively (Boemare *et al.,* 1993). Through natural openings of insect pests viz mouth, anus and spiracles or intersegmental membranes of insect cuticle in case of *Heterorhabditis,* the IJs enter the body of the insect (Bedding & Molyneux, 1982) and finally reach hemocoel. There the IJs release their mutualistic bacteria from their intestine. These bacteria release some enzymes, toxic substances and antibiotics to putrefy the body of the insect host and abet in making a nutrient soup of hemolymph that helps in the multiplication of bacteria and thus results in the killing of insect pests within 48 hours due to septicemia (Woodring & Kaya, 1988). Also in IJs venom, protein Pfam, proteases and protease inhibitors are profusely present. In the venom of *S. carpocapsae* 56% of the TIL a domain-containing protein is present (Lu *et al.,* 2017). After this whole process, the infective juveniles become feeding juveniles or third-stage juveniles that are functional and can feed on multiplying bacteria and degradative tissues of the host insect. Moulting rejiggers the feeding juvenile into the fourth stage juvenile and finally into adult. The adults after copulation lay eggs in the host medium where they hatch and develop into the first, second and third juvenile (infective juvenile) stages. If now the food supply is still there third juvenile stage develops into the fourth stage and then into adulthood and if the food supply gets exhausted the third stage juveniles or infective juveniles exit into the surrounding environment and are in search of a new host. So far as Steinernematids are concerned their IJs develop into amphimictic males and females whereas hermaphroditism is never a choice while as in the case of Heterorhabditids in a first generation, they always develop into hermaphrodites. These hermaphrodites will produce males, females and hermaphrodites in next generation (Dix *et al.,* 1992). Overall in general when the food supply is in plenty, three generations are produced within a host. In the host medium the eggs are laid where they hatch but in the case of hermaphrodites or older females, within the uterus eggs get hatched where they develop into feeding juveniles and start feeding on uterine tissues of the mother, a process called endotokia matricida (Johnigk & Ehlers, 1999), i.e., birth within the uterine tissue which ultimately leads to death of the mother.

HOST RANGE

EPNs attack and infect a broad spectrum of insects including *P. brassicae* and are experimented with for crop management throughout the world. It has been proved experimentally that these nematodes have the potential to infect over 200 species of insect pests (Woodring & Kaya, 1988), including *P. brassicae.* The ability to attack insect pests depends on a strategy of foraging viz cruising and ambushing (Campbell & Gaugler, 1997). An active and prompt cruising strategy depicted by *Heterorhabditis* sp. and *S. glaseri* (Levis, 2002), as they move through the soil very quickly, hence are most effective in managing insect pests. While ambushers are nictating and waiting while foraging. *S.*

carpocapsae and *S. scapterisci* are ambushers and for hours they nictate (Campbell & Gaugler, 1993). So in general host searching ability is stronger in the case of Heterorhabditids than in Steinernematids (Choo *et al.,* 1989).

MASS PRODUCTION

There are two different ways by which these nematodes are being produced viz. *in vivo* and *in vitro*.

The *in vivo* production method is one of the most and prolific methods so far as the quality of nematodes is concerned. Here, the EPNs are produced by the white trap method (White, 1927). The white trap is made of inverted watch glass that is placed in a Petri dish and a Whatman paper of fitting size is placed and with sterilized water, it is moistened. Around the watch glass sufficient amount of water is maintained. From the cadavers IJs get released, migrate to surrounding water and get entrapped there. From this water, IJs are harvested and collected in any suitable container. IJs produced by this method are very effective as per their virulence is concerned. For this method, *Galleria mellonella* or *Corcyra cephalonica* is generally used as these insects are very suitable and susceptible to EPNs (Woodring & Kaya, 1988).

In vitro production is the best method for their commercial production by using chicken, duck and Turkey offal medium impregnated in porous polyurethane foam (Bedding, 1984). Autoclaved plastic bags or glass flasks are used in this method as rearing containers which are inoculated by about 2000 IJs per gram of offal medium. By this technique on average from one bag or a flask of 500 ml capacity with 100 gram medium one billion IJs are produced. Some companies are there in some countries which are using this technique for producing EPNs commercially, e.g., Bionema in Sweden, Biologic in USA, Andermatt in Switzerland, Oviplant in Poland (Ehlers & Shapiro-Ilan, 2005). Also, different nematodes are commercially produced in different countries e.g., *S. carpocapsae* in the U.S.A. by the name of Ortho biosafe and Biovector similarly *S. feltiae* by the name of Manget in the U.S.A. and *H. bacteriophora* by the name of Nemasys in U.K.

APPLICATION TECHNOLOGY

Several factors are in a way to deter the delivery of IJs to be nearby or to adhere to the target insect pest for fruitful results. EPNs are live, tiny, tender and delicate so careful handling is a prerequisite by which adverse effects are minimized increasing the efficacy against the target organism and having a befitting activity and efficacy. The application of EPNs is mainly rendered to foliar and soil habitats. For best pest control and management the option of application equipment and the way nematodes are applied are critically important (Shapiro-Ilan *et al.,* 2006). For application of these mini creatures foliar or soil applications are two ways by which we can target them to insect pests. Regarding soil application hydraulic nozzles of large capacity are oftentimes required. Because nozzles with wide openings or orifices have comparatively the least shearing stress on them. A partial blockade in the orifice of the nozzle could be caused by any encumbrance such as any minute particle in the primary suspension (Gwynn *et al.,* 1999). Prior or post-irrigation is a prerequisite when EPNs are applied to the soil. By doing this the nematodes get proper assistance to go deep in the soil and tackle the target pest. Also by doing this nematodes get proper protection from environmental extremities as well (Ali *et al.,* 2005), but managing *P. brassicae* and the destruction caused by its soil application is not feasible as this pest is not present in soil. It is present on plants so foliar application is a must rather than soil application. For foliar application of nematodes spray distribution and droplet size are two vital considerations (Grewal, 2002). Flat fans and solid cone nozzles deposit a large quantity of IJs on the foliage which results in high mortality of target pest (Lello, 1996). Augmented deposition of EPNs on foliage will be achieved by accretion of adjuvants to the spray solution of EPNs. However, there is a limitation for foliar application as EPNs cannot thole UV radiation and extreme temperatures. The greater chances of success of application of EPNs can be achieved by using antidesiccants so that evaporation from EPNs solution on foliage can be deterred (Glazer & Novan, 1990). For better activity and survival on foliage, the addition of 10% glycerine has proved more effective (Nash & Fox, 1969).

EFFICACY OF ENTOMOPATHOGENIC NEMATODES AGAINST *P. brassicae*

A study was conducted in which three species of EPNs viz, *H. bacteriophora*, *S. thermophilum* and *S. glaseri* were tested against larvae of *P. brassicae* under laboratory conditions. All the species showed larvicidal activity but *S. thermophilum* caused mortality of larvae after 24 hours at 50 IJs/ larvae (Ramliana & Yadav, 2009). From native regions of Iran, EPNs viz *H. bacteriophora* and *S. feltiae* isolates were tested against the 4th and 5th instar larvae of *P. brassicae*. Both the isolates killed cabbage butterfly larvae, with LC_{50} values for *H. bacteriophora* at 25^{o}C and 30^{o}C against 4th instar larvae were 84.4 and 66.7 IJs/insect larvae respectively followed by *S. feltiae* which was less lethal than the former isolate (Abdolmaleki *et al.,* 2017). Virulence of EPN *H. pakistanensis* was conducted in lab conditions and it was seen that at 48h both 3rd and 4th instar larvae of cabbage butterfly were killed at an inoculum level of 200 IJs. Furthermore, this EPN in association with chemical insecticides is virulence

was augmented (Askary & Ahmad, 2020). In field and poly house conditions effectiveness of *H. bacteriophora* (EUPT-S26) local isolate caused 91.6 % mortality in poly house conditions when applied at 1500 IJs/ml and it was 94% in plots when the treatment level was 2000IJs/ml, regarding results of higher protection to plants this strain was used for field assays (Tomar *et al.,* 2022). *P. brassicae* is deemed as one of the dreaded pests of different crops, so laboratory experiments were conducted by using local EPN species viz., *S. borjomiense, S. tbilisiense, S. gurgistana, S. thesami* and *Heterorhabditis* sp. against *P. brassicae* at 100, 50 and 25 IJ levels against each insect. It was observed that all IJ levels have a high mortality rate. Among *Steinernema*, *S. thesami* resulted in 96.6% mortality most among *Steinernema* species and *Heterorhabditis* was most effective among all causing 97.5 % mortality. (Gorgadze *et al.,* 2018). Effect of EPNs viz., *S. feltiae, S. carpocapsae, S. asiaticum, S. pakitananese, S. glaseri, H. indica* and *H. bacteriophora* was observed on 4th instar larvae. The best two species *H. bacteriophora* and *S. glaseri* were taken and checked against 2nd, 3rd and 4th instar larvae of *P*. brassicae for their efficacy at 1500 IJs/ml after 48 hours, against all instar larvae, both nematode species were highly virulent and should be practised commercially (Abbas, 2021). In another study virulence of EPNs *H. bacteriophora, S. affine, S. feltiae* and *S. carpocapsae* against cabbage butterflies was observed in Turkey. The highest efficacy regarding mortality (84.33%) was observed in the case of *S. feltiae* at 25^{o}C (Yurt *et al.,* 2015).

CONCLUSION

Having in mind the above-reviewed literature it can be deemed that EPNs could prove a promising biocontrol agent in India as well as in the whole world as they prove lethal against every insect pest including cabbage butterfly. Chemical insecticides no doubt could prevent different insect pests including *P. brassicae* from damaging different crops including cole but due to several problems, e.g., resistance acquired by pests, different risks for humans, groundwater contamination, dwindling biodiversity as well as other environmental concerns. This void caused by chemical insecticides can be filled by microbial control agents. In the 21st century, sustainable agriculture depends on alternative options for managing pests. So for this to be achieved EPNs can provide significant and selective insect control. Tremendous work was done from different parts of the world on the effectiveness of EPNs against insect pests including *P. brassicae* but there is still a need to have considerable information on the potential lethality of different strains of EPNs. Regarding application technology foliar application could be developed up to best by using antideccicants and UV protectants. Also, these mini beneficial creatures require a low temperature for better shelf life. For this to achieve the final product becomes costly (Askary *et al.*, 2012). Hence more and more research should be endorsed to limit these requirements and make their formulations cost effective. Increased research is rather need of hour for optimizing stable formulations with higher shelf life and that would be easy to use. EPNs are produced commercially in different parts of the world but in India, only a few companies are producing EPNs. So there is a necessity to start and set up local-level cooperatives, cottage industries, and farmer entrepreneurship programmes to culture and produce EPNs that are cost effective and their better inclusion in Integrated Pest Management for suppression of insect pests including *P. brassicae*.

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