Innovative Entomopathogenic Strategies to Control Lepidoptera and Coleoptera to Sustain Agriculture

D.R.Saxena, F.A. Karim, A. N. Sheikh, N.J. Tupkar and L.V. Kharwade

Department of Zoology, Kamla Nehru Mahavidyalaya, Nagpur

saksenadevraj8465@gmail.com

ABSTRACT

Sustainable modern and organic farming will depend immensely on entomopathogen strategies to control outbreak of Lepidoptera, Coleoptera and other insect pests to prevent food losses in agriculture fields and in storage godowns; this is possible only when use of conventional insecticides is avoided for IPM, that has negative impact on environment, non-target organisms including humans, being non-cost effective but toxic, destructive to the soil microflora regulated natural nutrient cycle, decomposition cycle for recycling of nutrients in the ecosystem, etc. EPN aided control of insects and pathogens is totally ecofriendly it is an ecological bioengineering green technology for manipulating to maintain low populations of arthropods judiciously without exterminating non - harmful ones, even if they are non - agriculturally important.This will solve growing demand for qualitative and quantitative agriculture products for the ever increasing global populations for sustainable feeding, food security for all, poverty elimination, scarcity of food, controlling inflammation of food prices, zero hunger attainment, reduction in morbidity, human death, etc.The entomopathogens or the agriculture biologics like fungi, bacteria, nematode, viruses, and protozoa can be systematically and scientifically fully exploited to overcome above shortcomings by protecting agriculture, horticulture, forestry, etc., to harvest phytosanitary safe products. The usage and market demand of commercial entomopathogen based bio pesticides that are organism and species - specific is increasing globally to cope with global warming, El Nino, and climate change that trigger change in crop - patterns, land use, re-emergence of insect pests, etc.This necessitates changes in formulating suitable global policies for sustainable development.

Key Words: Lepidoptera, Coleoptera, entomopathogens, food sustenance, crop protection, climate change.

INTRODUCTION

The various crop pathogens and pests are responsible for worldwide estimated decline in yields and quality of wheat 21.5 %, rice 30 %, maize 22.5 %, potato 17.2 % and soybean 21.4 % (Savary et al., 2019).The loss can be minimized using either entomopathogens only or in combination with pesticides on rotation basis. More research is needed on interaction between pest and entomopathogens in relation to plants.

Natural enemies of the insects are the entomopathogens, predators, and parasitoids are used in IPM. Codling moth Cydiapomonella, spread to N. America in 1750 (Slingerland, 1898)

and is a devastating pest of Apple, pear and walnuts in most countries (Barnes, 1991).Three to seven days post hatching neonate larvae devour the fruit and pass through 5 - larval instars to emerge from fruit (Beers et al., 1993).Nearly 60% of diseases un insects develop due to pathogenic fungi (Vega et al., 2009); entomopathogenic fungi life cycle have seven stages and 3 - 7 days post infection the insect dies (Hajek et al., 2007; Xiong et al., 2012; Gillespie et al., 2004 and Humber, 1991). The most harmful agricultural pest is Helicoarmigera (Lepidopteran, Noctuidae) a polyphagous moth on cotton, maize, chickpea, tomato, sorghum, sunflower, etc., is widespread in Europe, Asia, Africa, Oceania (Akbulut et al., 2003).

The white grub larvae of Coleoptera (Scaraboeidae) feed on turf grass, pasture and horticultural vegetation in most parts of the world.Important beetle species are Melolonthamelolontha, Phyloperthahorticola (Jackson and Klein, 2006). The entomopathogenic fungi groups Entomophthorales in the temperate forests (Burges, 1981) and Hypocreals in the humid tropic (Kram and Kram, 2012) distributed in Arctic Circle and Antarctica (Evans, 1982). The 17 entomopathogen Cordyceps species was recorded mostly on larvae and pupae of hepialid Lepidopterans like Glossata, Exoporia and Hepialidae by Shrestha et al., (2016) and Baral et al., 2015.

Coleoptera is the most species rich group includes families Scarabaeidae, Geotrupidae (boring dung beetle), Lucanidae (stag beetle) (Slipinski et al., 2011). Super families like Scarabaeoidea, Elaleroidea, Chrysomeoidea, Cucuyoidea, Curculionidae, Tenbrionidae, Staphylinoidea and Scaraboidea (Slipinski e et al., 2012). Lepidoptera is amongst the largest orders (Gaston, 1991) with 160, 000 spp., four orders, 45 superfamilies and 139 families (Van, 2011; Wagner, 2001).The largest Suborder Glossata comprise of all known 99.9% of Lepidopterans that become infected with Cordyceps and allied genera of 22 species of entomopathogenic fungi (Shrestha et al., 2016; Baral et al., 2015); the host range of Cordyceps is broader on many orders of Lepidopteran but include more than 95 % of butterflies and moths and the Coleopteran beetle include mostly larvae; the rest are adults or pupae. Nearly 60% of species of Cordyceps infect Coleoptera and Lepidoptera. Ophiocordyceps sinensis is a fungal entomopathogen abundantly collected (Deokota, 2008; Winkler, 2008; Shrestha and Bawa, 2013; Shrestha and Bawa, 2014), because it's market value is double that of gold by weight and is referred to as Himalayan Gold (Gould, 2007), Tibetan Gold or Biological Gold (Shrestha and Bawa, 2014) due to their medical applications The Colorado potato beetle, Leptinotarsa decemlineatus (Say) and the diamond back moth, Plutellaxylotella (L.) have developed resistance against insecticides, and both are pests of potatoes as well as Cruciferous crops (Alyokhin et al., 2008; Wang et al., 2021).

Fungal entomopathogens:

Overwintering codling moths harbour multitude of fungal species (Jaques and McClellan, 1968; Hagley, 1971; Labanowski, 1981; Glen, 1982; Subinprasaret, 1987and Zimmermann and Weiser, 1991).Extensive researches have been already done regarding biological potential of Beauveria bassiana as a control agent (Ferron and Vincent, 1978; Falcon and Huber, 1991; Cross et al., 1999 and Garcia Gutierrez et al., 2004). In each of the trees applied with 6×10^9 conidia

50% mortality of mature emerging Codling moth larvae from such trees was reported by Ferron and Vincent (1978).Effective control of neonate Codling moth larvae was achieved with a native strain BbP1 and Mycotrol and Meta - Sin that are two commercial products of B.bassiana at 1.2 x 10^{12} conidia/hectare.EPF have a wider host range for fossorial and terrestrial inhabiting insects in comparison to EMV and EMB that have high and moderate host specificity respectively. EMF produce secondary metabolites or bioactive toxins like cyclosporins, cytochalasins, beauvericin, beauverolides, etc., under optimal environmental conditions grow into germ tubes and appressosorium and they pierce insect cuticle into insect body. Inside the tissues hyphae proliferate to produce fresh generation of conidia to infect fresh insects (Quesada et al., 2020).

Bacterial entomopathogens:

The general Bacillus, eg., the bacteria Clostridium and Paenbacillus form spores; while the generasPsuedomonas and Photorhabdus, Xenorhabdus, Serratia and Yersinia do not form spore but are entomopathogen when taken in with food. Bt.sub.sp. tenebrionis (Btt) kill Coleopterans; Btk and Bt. sub sp. aizawai (Bta) is effective in biocontrol of caterpillars.Bt is a gram-positive soil bacterium produces a parabasal body comprised of crystals and delta-endotoxins or Cry protoxin proteins during sporulation and when consumed get activated to result in pore-formation in the mid gut lining cells eventually leaking the gut content in to the haemocoel and haemolymph enter in gut lumen (Fig.1). The haemolymph pH alter cause septicemia that is lethal to Lepidoptera butterflies and Coleoptera beetles that get infected with the bacterium (Ben-Dov, 2014 and Schiinemannet al., 2014).

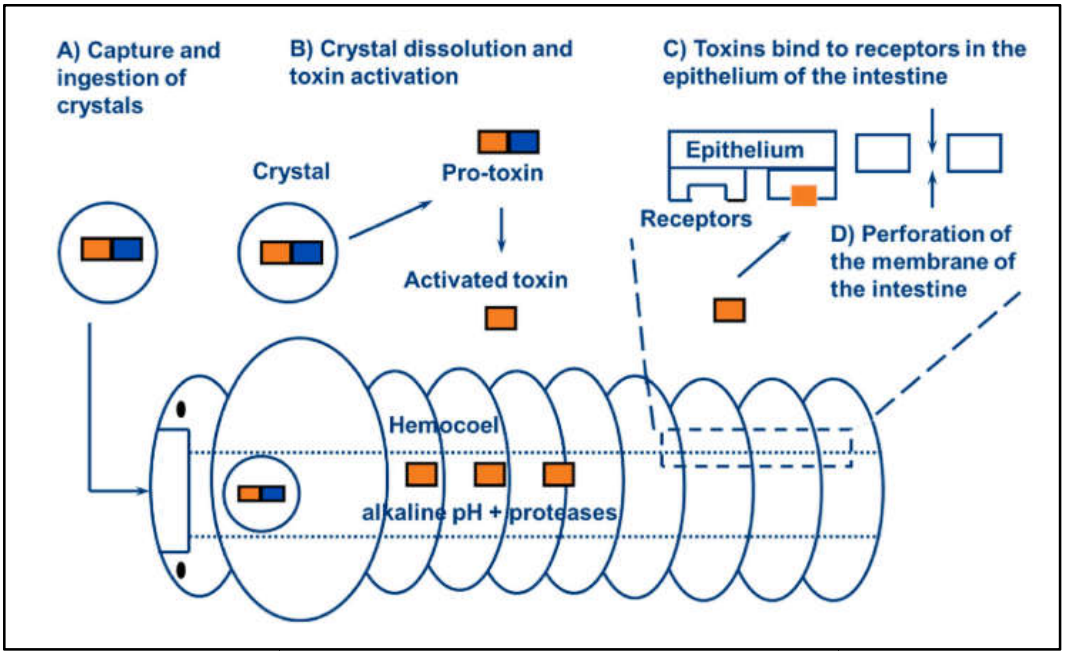

Fig.1. Schematic diagram showing the mode of action of Bacillus thuringiensis in Lepidoptera (Sabbahi et al., 2022)

Nematode entomopathogens:

Natural population of Codling moths carry nematodes of families Mermithidae and Steinernematiidae (Poinar, 1991); Steinernemecarpocapsae infect the Codling moth larvae gradually at low temperature of 15^0C and cease when temperature is 10^0C (Lacey and Unruh, 1998 and Vega et al., 2000). The S.carpocapsae possess limited host searching behavior i.e an ambusher species (Lewis et al., 1995 and Campbell and Gaugler 1997) that controls the cocooned Codling moth larvae at optimum temperature and moisture conditions. According to Lacey et al., (2005b) the combination of Silwet L77 wetting agent and Stockosorb humectant with 10 S.feltiae entomopathogenic infective juveniles per ml (called "Dauer stage") that releases symbiotic bacteria which kill and digest host tissue; at low and high moisture 92-95 % mortality of cocooned Codling moth against 46-57 % mortality at the same IJ concentration was observed in the absence of adjuvant (Fig. 2).

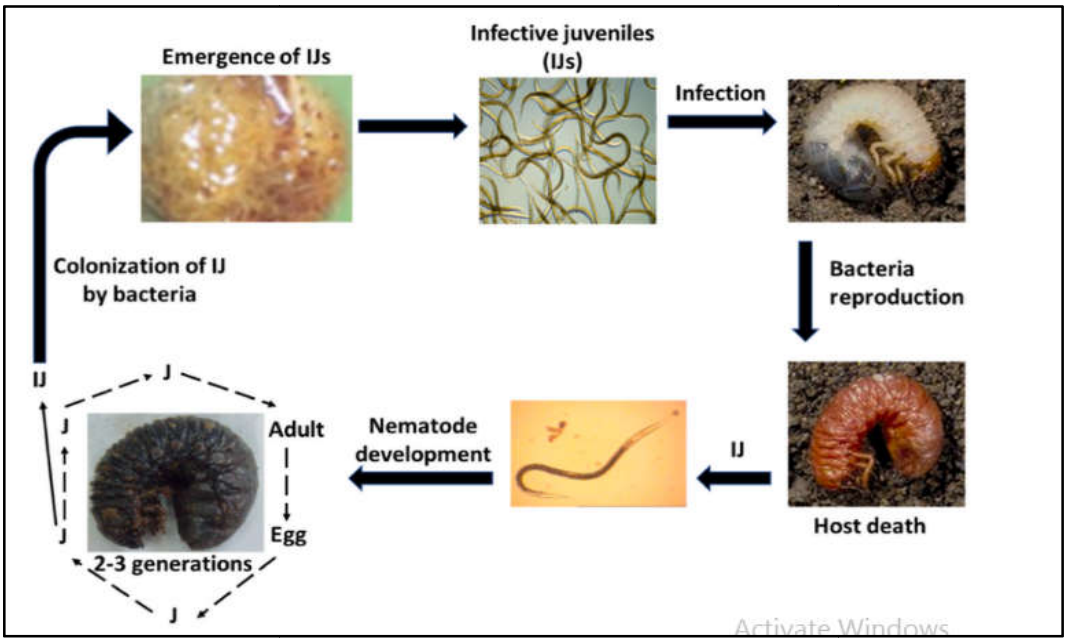

Fig. 2. Life cycle of entomopathogenic nematode (Sabbahi et al., 2022)

Viral entomopathogens:

Lepidopteran caterpillars with chewing mouth parts are killed by entomopathogen viruses that enter their gut along with plant food. The ds DNA Baculoviruses infect and destroy several species of insect pests by forming crystalline occlusion bodies.Granuloviruses and Nucleopolyhedroviruses are Baculoviruses (Ruiu, 2018).When ingested the virion coat protein dissolve and set free more occlusion derived viruses in the larval stomach to infect the microvilli of epithelial cells.The other structurally different but genetically identical viral form denoted as "budded viruses" is produced in the nucleus of midgut cells and spread infections into other cells (Wang et al., 2019) and facilitate further proliferation throughout the host body to eventually lyse it. The Codling moth, Cydiapomonellagranulovirus; the beet army worm Spodoptera exigua

multiple nucleopolyhedrovirus and the corn earworm called Helicoverpazea are the single envelope viruses marketed as the baculovirus based products for control of the host insects in which they occur naturally.

Protozoan entomopathogens:

Microsporodian single celled eukaryotes are obligate intracellular pathogenic protists related to fungi (Solter et al., 2012) and on ingestion insects get infected, but parasitoids and predators also spread microsporidians to several insect populations (Sarwar et al., 2021). Noctuid decides carry the microsporidia the Vairimorphanecatrix, this host-pathogen interactions on target pest population have been studied (Solter et al., 2012). Nosema spore germinate in the hosts midget after ingestion and infection spreads to other tissues and organs later leading to disintegration and septicemia, thus slow moving diseases or endodontics weakening affect growth of host and fecundity declines (Bjornson and Oi, 2014) and infection is vertically transmitted to the next generation (Solter et al., 2017).

Herbivore insects damage approximately $1/5^{th}$ of the world total crop produced annually. Long term pest management involves release of exotic microorganisms but only after rigorous research and permission from government authorities (Grzywacz et al., 2014; Ramirez-Guzman et al., 2018).

Commercialization of EPS involves either in vitro culture of nematodes, fungi and bacteria or the in viva culture of nematodes and viruses (Deka et al., 2021).The mechanism of action of entomopathogens must be evaluated thoroughly to avoid risk from them to non - target organisms (Reinbacher et al., 2021). Currently, globally nearly 209 registered commercial microbial isolates from 94 species are available for pest management (Lentern et al., 2018). Microbial bio control agents are produced by approximately 220 manufacturers (IBMA-glob 2022). The projected biopesticide market estimated in Northern USA for 2022 was US $ 1.67 billion (Arthur's et al., 2019). Nearly 390 biopesticide registered products exist in U.S. market (O.UsEpa 2022).The toxic mode of action of the various microbial biopesticides prove lethal to insect pests: affect the neuromuscular tissues, inhibit growth stages of life cycle, exhibit non-specific multi-site inhibition, impair or poison the metabolic pathways and also impair the gut physiology (Castagnola and Stock 2014). The insect pests (Lepidopteran, Coleoptera, etc.,) of crops, fruits, vegetables, etc., inhabit bizarre sites of trees like the bark, cone, root, stem, fruit, seed, etc., limit their control after application of multitude of EP's, as most or nearly EP's are species specific and are commercially expensive. Moreover, the short shelf life and the influence of environmental factors in various parts of the globe on these insect pests life cycle are constraints in the limited use of microbial biopesticides. Extensive research on formulation of a single EP biopesticide solely effective against one group of say Lepidopteran or Coleopteran (effective against all its species inclusive) is needed with wide usage across the globe, as the future survival of human civilization rests on agriculture and to some extent on animal husbandry. Dry unprocessed and processed foods of plant and animal origin will sustain human lives in the scenario of global climate change, the worst sufferers will be India, Africa, etc., the developed nations would not be spared by this effect (food ratio of 65 % agriculture: 35 %

animal husbandry; formula concept for dry processed and unprocessed foods with enhanced shelf life, proposed by D.R. Saxena, 2023).

Conclusion

Alternative novel crop protection strategies are a bioethical substitute to chemical methods of insect pest management practices globally and regionally that is a "Nongreen Technology". Entomopathogens like Bacillus, Beauveria, Nematodes (Heterorhabditisetc.), Protozoans, Fungi, Parasitic plants, Viruses, etc., in the form of soil spray, seed treatment, foliar spray can be applied for sustenance agriculture of cereal grains, pulses, oil seeds, fruits and vegetables.Microbial pesticides either alone or as various combinations as moist powder, liquid, and granular are to be experimented; some single microbial formulations are currently available in the Agromarkets.Hence, more specific/target crop oriented research on new entomopathogens must be discovered in this "Age Of Climate Change".A compound (2 or more or all entomopathogens combined) microbial pesticide called "Universal Microbial pesticide" in the form of spray, powder, granular ball is hypothesized that may have capacity to wipe out insects that damage food crops, fruits and vegetables (hypothesis proposed by D.R.Saxena et al., 2019).

References

Akbulut S, Yuksel B and Keten A. The Lepidoptera (INSECTA) Fauna of Düzce Province, Turkey. Turkish Journal of Zoology. 2003; 27(4): 257-268.

Alyokhin, M. Baker, D. Mota-Sanchez, G. Dively, E. Grafius, Colorado potato beetle resistance to insecticides, Am. J. Potato Res. 85 (2008) 395–413, https:// doi.org/10.1007/s12230-008-9052-0.

Arthurs, S., S.K. Dara, Microbial biopesticides for invertebrate pests and their markets in the United States, J. Invertebr. Pathol. 165 (2019) 13–21, https://doi. org/10.1016/j.jip.2018.01.008.

Augustyniuk-Kram A. and K. J. Kram, “Entomopathogenicfungi as an important natural regulator of insect outbreaks inforests(review),”inForestEcosystems—MorethanJustTrees,J. A.BlancoandY.-H.Lo,Eds.,chapter12,pp.265–294,InTech,Rijeka,Croatia,2012.

Baral, B., B. Shrestha, and J. A. T. da Silva, “A review ofChinese Cordyceps with special reference to Nepal, focusing onconservation,”EnvironmentalandExperimentalBiology,vol.13,no.2,pp.61–73,2015.

Barnes, M. M. 1991. Tortricids in pome and stone fruits, codling moth occurrence, host race formation and damage, pp. 313-327. In: L.P.S. van der Geest and H.H. Evenhuis (eds), Tortricid Pests, Their Biology, Natural Enemies and Control. Elsevier Science Publishers, Amsterdam, The Netherlands.

Beers, E. H., J. F. Brunner, M. J. Willett & G. M. Warner. 1993. Orchard Pest Management: A Resource Book for the Pacific Northwest: Good Fruit Grower, Yakima, WA.

Ben-Dov, E. Bacillus thuringiensis subsp. israelensis and its Dipteran-Specific Toxins, Toxins 6 (2014) 1222–1243, https://doi.org/10.3390/toxins6041222

Bjørnson, S. D. Oi, Microsporidia biological control agents and pathogens of beneficial insects, in: Microsporidia, John Wiley & Sons, Ltd, 2014, pp. 635–670, https://doi.org/10.1002/9781118395264.ch25.

Burges, H. D. & K. A. Jones. 1998. Formulation of bacteria, viruses and Protozoa to control insects, pp. 34-127. In: H. D. Burges (ed.), Formulation of Microbial Biopesticides. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Campbell, J. F. & R. R. Gaugler. 1997. Interspecific variation in entomopathogenic nematode foraging strategy: dichotomy or variation along a continuum? Fund. Appl. Nematol., 20: 393-398.

Castagnola, A., S.P. Stock, Common virulence factors and tissue targets of entomopathogenic bacteria for biological control of Lepidopteran pests, Insects 5 (2014) 139–166, https://doi.org/10.3390/insects5010139.

Cross, J. V., M. G. Solomon, D. Chandler, P. Jarrett, P. N. Richardson, D. Winstanley, H. Bathon, J. Huber, B. Keller, G. A. Langenbruch& G. Zimmermann. 1999. Biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. Biocontr. Sci. Technol., 9: 125-149.

Deka, B., C. Baruah, A. Babu, Entomopathogenic microorganisms: their role in insect pest management, Egypt, J. Biol. Pest Control 31 (2021) 121, https://doi. org/10.1186/s41938-021-00466-7.

Devkota, S. "Approach towards the harvesting of Cordycepssinensis (Berk.) Sacc. in pastures of Dolpa, Nepal," in MedicinalPlantsinNepal:AnAnthologyofContemporaryResearch,P.K.Jha,Karmacharya, S.B.,M.K.Chetri,C.B.Thapa,andB. B. Shrestha, Eds., pp. 90–96, Ecological Society (ECOS),Kathmandu,Nepal,2008.

Evans, H. C. "Entomogenous fungi in tropical forest ecosys-tems:anappraisal,"EcologicalEntomology,vol.7,no.1,pp.47–60,1982.

Falcon, L. A., & J. Huber. 1991. Biological control of the codling moth, pp. 355-369. In: L.P.S. van der Geest& H. H. Evenhuis (eds.), Tortricid Pests, Their Biology, Natural Enemies and Control. Elsevier Science Publishers, Amsterdam, The Netherlands.

Ferron, P. 1978. Biological control of entomogenous pests by entomogenous fungi. Annu. Rev. Entomol., 23: 409-442.

Garcia-Gutierrez, C., M. B. GonzalesMaldonado, H. Medrano-Roldan & I. Chairez-Hernandez. 2004. Evaluación de la cepa BbP1 de Beauveria bassiana, Mycotrol®, Meta-Sin® y azinfos-metilico contra Cydiapomonella L. (Lepidoptera: Tortricidae) enlaboratorio y campo. Folia Entomol. Mex., 43: 1-7.

Gaston, K.J."Themagnitudeofglobalinsectspeciesrichness,"ConservationBiology,vol.5,no.3,pp.283–296,1991.

Gillespie AT, Bailey AM, Cobb B, Vilcinskas A. Fungi as elicitors of insect immune responses. Archives of Insect Biochemistry and Physiology. 2000; 44: 49-68.

Glen, D. M. 1982. Effects of natural enemies on a population of codling moth, Cydiapomonella. Ann. Appl. Biol., 101: 199-201.

Gould, R. "Himalayan Viagra, Himalayan Gold? Cordycepssinensis brings new forces to the Bhutanese Himalaya," TropicalResourcesBulletin,vol.26,pp.63–69,2007.

Grzywacz, D., D. Moore, R.J. Rabindra, in: J.A. Morales-Ramos, M.G. Rojas, D. I. Shapiro-Ilan (Eds.), Chapter 15 - Mass Production of Entomopathogens in Less Industrialized Countries, Mass Prod. Benef. Org., Academic Press, San Diego, 2014, pp. 519–561, https://doi.org/10.1016/B978-0-12-391453-8.00015-7.

Hagley, E. A. C. 1971. The occurrence of fungal diseases of the codling moth in unsprayed apple orchards in Ontario. Proc. Entomol. Soc. Ontario, 101: 45-48.

Hajek, A.E., M.S. Goettel, Guidelines for evaluating effects of entomopathogens on non-target organisms, in: L.A. Lacey, H.K. Kaya (Eds.), Field Man. Tech. Invertebr. Pathol. Appl. Eval. Pathog. Control Insects Invertebr. Pests, Springer Netherlands, Dordrecht, 2007, pp. 816–833, https://doi.org/10.1007/978-1-4020-5933-9_40.

Humber RA. Fungal Pathogens of Aphids. Proceedings of an OSU Centennial Event. 1991.

IBMA, Home page, IBMA-glob. https://ibma-global.org/, 2022. (Accessed 23 May 2022).

Jackson, T. A., and M. G. Klein (2006). Scarabs as pests: a continuing problem. Coleopts. Bull. 60, 102-119.

Jaques, R. P. & C. R. MacLellan. 1968. Fungal mortality of overwintering larvae of the codling moth in apple orchards in Nova Scotia. J. Invertebr. Pathol., 7: 291-296.

Labanowski, G. S. 1981. The pathogens and parasites recovered from the hibernating larvae of the codling moth, Laspeyresiapomonella (L.) (Lepidoptera, Tortricidae). Pol. Pis. Entomol., 51: 163-170.

Lacey, L. A., L. G. Neven, H. L. Headrick & R. Fritts, Jr. 2005b. Factors affecting entomopathogenic nematodes (Steinernematidae) for control of overwintering codling moth (Lepidoptera: Tortricidae) in fruit bins. J. Econ. Entomol., 98: 1863-1869.

Lewis, E. E., P. S. Grewal & R. Gaugler. 1995. Hierarchical order of host cues in parasite foraging strategies. Parasitology, 110: 207-213.

Poinar, G. O., Jr. 1991. Nematode parasites, pp. 273-281. In: L. P. S. van der Geest& H. H. Evenhius, (eds.), Tortricid Pests, their Biology, Natural Enemies and Control. Elsevier Science Publishers, Amsterdam, The Netherlands.

Quesada Moraga, E., Entomopathogenic fungi as endophytes: their broader contribution to IPM and crop production, Biocontrol Sci. Technol. 30 (2020) 864–877, https://doi.org/10.1080/09583157.2020.1771279.

Ramírez-Guzman, K.N., C. Torres-Leon, ´ E. Martínez-Terrazas, R. De la CruzQuiroz, A.C. Flores-Gallegos, R. Rodríguez-Herrera, C.N. Aguilar, Chapter 7 - biocontrol as an efficient tool for food control and biosecurity, in: A. M. Grumezescu, A.M. Holban (Eds.), Food Saf. Preserv., Academic Press, 2018, pp. 167–193, https://doi.org/10.1016/B978-0-12-814956-0.00007-X.

Reinbacher, L., S. Bacher, E. Praprotnik, G. Grabenweger, Standard non-target tests for risk assessment of plant protection products are unsuitable for entomopathogenic fungi—a proposal for a new protocol, J. Soils Sediments 21 (2021) 2357–2368, https://doi.org/10.1007/s11368-021-02919-w.

Ruiu, L. Microbial biopesticides in agroecosystems, Agronomy 8 (2018) 235, https://doi.org/10.3390/agronomy8110235.

Ślipiński, S.A., R.A.B.Leschen,andJ.F.Lawrence,"Order Coleoptera Linnaeus, 1758," in Animal Biodiversity: An OutlineofHigher-LevelClassificationandSurveyofTaxonomicRichness,Z.Zhang, Q.Ed.,vol.3148,pp.203–208,Zootaxa,2011.

Sabbahi, Rachid., Hock, Virginia., Azzaoui, Khalil., Saoiabi, Sanaa., Hammouti, Belkheir. (2022). A global perspective of entomopathogens as microbial biocontrol agents of insect pests. Journal of Agriculture and Food Research. 10.100376.10.1016/j.jafr.2022.100376.

Sarwar, M., N.A. Shad, R. Batool, Entomopathogenic Protozoa roles in the management of insects pest populations, in: Biopestic. Org. Farming, CRC Press, 2021.

Savary, S., L. Willocquet, S.J. Pethybridge, P. Esker, N. McRoberts, A. Nelson, The global burden of pathogens and pests on major food crops, Nat. Ecol. Evol. 3 (2019) 430–439, https://doi.org/10.1038/s41559-018-0793-y.

Schünemann, R., N. Knaak, L.M. Fiuza, Mode of action and specificity of Bacillus thuringiensis toxins in the control of caterpillars and stink bugs in soybean culture, ISRN Microbiol (2014), e135675, https://doi.org/10.1155/2014/ 135675, 2014.

Shrestha, B. "Yarsagumba: fungal biology and medicinal value,"inEnvironmentandNaturalResources,P.K.Jha,F.P.Neupane, M. L. Shrestha, and I. P. Khanal, Eds., pp. 607–610, NepalAcademyof Science andTechnology,Khumaltar,Nepal, 2013.

Shrestha, B., E. Tanaka, J.-G. Han et al., "A Brief chronicle of thegenusCordycepsFr.,theoldestvalidgenusinCordycipitaceae(Hypocreales,Ascomycota),"Mycobiology,vol.42,no.2,pp.93–99,2014.

Shrestha, B., W. Zhang, Y. Zhang, and X. Liu, "What is theChinese caterpillar fungus Ophiocordyceps sinensis (Ophio-cordycipitaceae)?"Mycology,vol.1,no.4,pp.228–236,2010.

Shrestha, S., A.K.Shrestha,J.H.Parketal.,"Reviewonpharma-cologicallyactivemetabolitesfromYarsagumba(Ophiocordy-cepssinensis),anepitomeofHimalayanelixir,"NepalJournalofScienceandTechnology,vol.14,no.2,pp.49–58,2013.

Shrestha, S., B.Shrestha,J.H.Park,D.Y.Lee,J.G.Cho,andN.I.Baek,"ChemicalconstituentsofYarsagumba(Ophiocordycepssinensis (Berk.) Sung et al.), a valued traditional Himalayanmedicine,"NepalJournalofScienceandTechnology,vol.13,no.1,pp.43–58,2012.

Shrestha, U. B. and K. S. Bawa, "Economic contribution ofChinesecaterpillarfungustothelivelihoodsofmountaincommunities in Nepal," Biological Conservation, vol. 177, pp.194–202,2014.

Slingerland, M. V. 1898. Codling moth in New England in 1750. New York Agr. Expt. Sta. Bull. (Cornell), 142: 85-155

Solter, L.F., A.E. Hajek, L.A. Lacey, Chapter 2 - exploration for entomopathogens, in: L.A. Lacey (Ed.), Microb. Control Insect Mite Pests, Academic Press, 2017, pp. 13–23, https://doi.org/10.1016/B978-0-12-803527-6.00002-0.

Solter, L.F., J.J. Becnel, D.H. Oi, Chapter 7 - microsporidian entomopathogens, in: F.E. Vega, Kaya, H.K. (Eds.), Insect Pathol, second ed., Academic Press, San Diego, 2012, pp. 221–263, https://doi.org/10.1016/B978-0-12-384984-7.00007-5.

Solter, L.F., J.J. Becnel, D.H. Oi, Chapter 7 - microsporidian entomopathogens, in: F.E. Vega, Kaya, H.K. (Eds.), Insect Pathol, second ed., Academic Press, San Diego, 2012, pp. 221–263, https://doi.org/10.1016/B978-0-12-384984-7.00007-5.

Subinprasert, S. 1987. Natural enemies and their impact on overwintering codling moth populations (Laspeyresiapomonella L.) (Lep., Tortricidae) in South Sweden. J. Appl. Entomol., 103: 46-55.

UsEpa, O.Biopesticideactiveingredients(accessedJune13,2022),https://www.epa.gov/ingredients-used-pesticide-products/biopesticide-active-ingredients,2020.

Van Lenteren, J.C., K.Bolckmans, J. Kohl, ¨ W.J. Ravensberg, A. Urbaneja, Biological control using invertebrates and microorganisms: plenty of new opportunities, BioControl 63 (2018) 39–59, https://doi.org/10.1007/s10526- 017-9801-4.

Vega, F. E., L. A. Lacey, A. P. Reid, F. Herard, D. Pilarsa, E. Danova, R. Tomov & H. K. Kaya. 2000. Infectivity of a Bulgarian and an American strain of Steinernema carpocapsae (Nematoda: Steinernematidae) against codling moth. BioControl, 45: 337-343.

Wagner, W., Möhrlen, F., and W. Schnetter (2002). Characterization of the proteolytic enzymes in the midgut of the European Cockchafer, Melolonthamelolontha (Coleoptera: Scarabaeidae). Insect Biochem. Molec. Biol. 32, 803-814.

Wang, J., X. Zheng, J. Yuan, S. Wang, B. Xu, S. Wang, Y. Zhang, Q. Wu, Insecticide resistance monitoring of the diamondback moth (Lepidoptera: plutellidae) populations in China, J. Econ. Entomol. 114 (2021) 1282–1290, https://doi.org/ 10.1093/jee/toab027.

Wang, M., Z. Hu, Cross-talking between baculoviruses and host insects towards a successful infection, Philos. Trans. R. Soc. B Biol. Sci. 374 (2019), 20180324, https://doi.org/10.1098/rstb.2018.0324.

Winkler, D.,"YartsaGunbu(Cordycepssinensis)andthefungalcommodificationofTibet'sruraleconomy,"EconomicBotany,vol.62,no.3,pp.291–305,2008.

Xiong Q, Xie Y, Zhu Y, Xue J, Li J, Fan R. Morphological and ultrastructural characterization of Carposinasasakii larvae (Lepidoptera: Carposinidae) infected by Beauveria bassiana (Ascomycota: Hypocreales: Clavicipitaceae). Micron. 2012; 44: 303-311.

Zimmermann, G. & J. Weiser. 1991. Pathogens and diseases, pp. 253-271. In: L. P. S. van der Geest& H. H. Evenhuis (eds.), Tortricid Pests, Their Biology, Natural Enemies and Control. Elsevier Science Publishers, Amsterdam, The Netherlands.