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Pre-feasibility Study Establishment of Galleria Farm for Entomopathogenic Nematodes (EPNs) Bio-Insecticides.

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ABSTRACT

The development of indigenous isolates of entomopathogenic nematodes (EPNs) in vivo is a point of concern for small and mediumsized businesses (SMBs), smaller family farms, greenhouse cultivation, or even a cottage industry. Although EPN research studies in Egypt aim to the identification and usage of highly potent and effective indigenous strains against economically significant insect pests, no companies have vet been established for the development and sale of these plant protection products except for small special units affiliated with some research institutes and universities. On increasing demand for nonchemical forms of pest control and the high price product of EPNs, this research assessed the financial feasibility of Galleria mellonella farming for in vivo production of EPNs at small and medium scales. The main finding indicated that the cost production of indigenous EPNs (one Sachest containing 5 x 10^7 IJs) is 200 LE which is considered a low-price product in comparison to the price of products which is variable from about US\$ 15 to US\$ 160 per sales unit. Moreover, the capital recovery period after one year is 99.7 %.

INTRODUCTION

Entomopathogenic nematodes (EPNs) have specific biological and ecological characteristics that made their use in biological control remarkably safe for the environment besides the wide host range and the compatibility with other agrochemicals (Vashisth, 2013), so work on EPNs shouldn't be limited to research but also stimulated the industry to work together for farm-oriented output (Lacey *et al.*, 2015). EPNs have not yet seen widespread commercialization, in contrast to other microbial biocontrol agents. The methods and ideas used to turn a nematode into a commercial product are comparable to those used in the creation of a bacterial bioagent commercial product. Contrary to microorganisms, multicellular organisms have commercially undesirable traits like short shelf lives, a lack of environmentally resistant life stages, typical asexual reproduction with brief life cycles, etc.; as a result, they require special procedures during production, shelf-life, packaging, storage, transport, and delivery systems, which raises the cost of the product (Thakur *et al.*, 2020).

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Due to this, the commercial usage of EPN products and the advantages they offer to farmers remain restricted, despite their enormous potential. Mass production of EPNs can be *in vivo* or *in vitro* for research studies or for commercialization (Morales-Ramos *et al.*, 2013).Large-scale commercial yields of EPNs require economies of scale(i.e., decreasing costs in yields with an increasing scale of operation) (Friedman, 1990). Without such economies of scale, the commercial production of EPNs will be confined to cottage industries (small-scale decentralized manufacturing businesses often operated out of a home rather than a purpose-built facility, the labor force consists of family units or individuals working at home or their own farm with their own equipment)(Kusuma, 2021).

EPNs developed primarily for biocontrol are grown *in vitro* (mostly in liquid culture but also to some extent in solid fermentation). However, *in vivo* EPN production is generally employed for lab research and small-scale efficacy tests in greenhouses or fields. Compared to *in vitro* methods, *in vivo* methodologies have a lower economy of scale due to the costs of labor and the costs of insect hosts required for infection. Nonetheless, small companies continue to produce EPNs using *in vivo* technology for commercial application (Saleh *et al.*, 2020). *In vivo* manufacturing is straightforward, necessitating little infrastructure investment and yielding good quality nematodes with extended shelf lives, Also, Advances in the mechanization of *in vivo* methodology and insect host production have led to improvements in efficiency.

Early researchers on the manufacture and commercialization of EPNs have discovered that the costs, the timely availability of host insects, and the length of time needed for each production cycle limit in vivo production. However, this production method is best suited for countries that have low labour costs or for serving high-value markets. Also, keeping a continuous supply of the insect host through mass rearing support their availability (Nagesh et al., 2017). Despite limited research on the financial feasibility of insect farming, data regarding the financial feasibility and profitability of insect farming and their products is important for investors, policymakers and other stakeholders to be able to assess policy and regulations for this emerging sector that wishes to supply new markets. Small and medium-sized businesses (SMBs) have limited available information, which is very critical for entrepreneurs to launch new businesses or expand existing ones or scale up their ongoing business. These reference data are currently only limited and available for insect companies, and therefore there is a need for reliable economic data to contribute to building such a framework for the insect pest biocontrol sector. This will improve the quality of the risk assessment and also credit access for insect farmers (Madau et al., 2020).

In this context, we establish a preliminary feasibility study before starting the project, meaning a study of the idea of establishing a farm called the Galleria farm at small and medium-sized scale to rear *Galleria mellonella*, the host larvae in order to maintain a constant supply of *G. mellonella* larvae for *in vivo* production of low cost, native isolates of EPNs in a trial to scale- up EPNs *in vivo* production which would be a vital step towards their implementation in Egypt. Also, the current study used simple and cost-effective tools and methods for rearing *G. mellonella* and production of EPNs using only basic equipment which is accessible to the cottage industry and small farmers. Besides, the study indicated the costs and sales prices and analyzed the expected farm profitability.

MATERIALS AND METHODS

Project Financing:

A pre-feasibility study has two main tasks, the first task is to produce *G. mellonella* (unit A), and the second task is to produce, native isolates of EPNs (Unit B) and that's the main goal. In this study, all manufacturing expenses for a year are calculated. The following

inputs were taken into account: Firstly, the fixed assets in the form of machinery & Utility. Second, the working capital is represented by the raw materials (variable cost) like food and others/year, besides salaries and overheads. Other measurements like Fixed Capital (FC), Working Capital (WC), Total invested capital = F.C. + W.C., Other fixed costs for a year, Depreciation and value of year profit = (Value of year production – Other fixed costs) were calculated.

The Measurements Listed Below Were Used to Evaluate The Obtained Data According to Zhang, (2005):

Fixed Capital (F. C.). ii- Working Capital: (W. C.). iii- Total invested capital = F.C. +
 W.C. iv- Other annual fixed costs. v- Depreciation. vi- Year Profit Value is calculated as production for the year less other fixed costs.

vii- Percentage of profit = $\frac{(Value \ of \ year \ profit)}{Total \ invested \ capital} \times 100$

viii-Capital recovery period = $\frac{(Value \ of \ year \ profit)}{Value \ of \ year \ profit + Depreciation} \times 100$

Basic Approaches for *G. mellonella* **Mass Rearing (Unit A): Diet Preparation:**

G. mellonella larvae are mass-reared using an artificial diet consisting of 10% of glycerol, 10% of organic honey, then, 40% of maize flour and Powdered milk 10%, all components were mixed with the liquid until the mixture crusted; then, (10%) instant dry baker's yeast was added and mixed thoroughly. The culture medium was prepared fresh and can be stored in the refrigerator enclosed in a plastic bag or any closed container to avoid drying, each rearing container needs about 0.25 Kg which is renewed three times during the month. The following artificial diet was according to Van Zyl & Malan (2015) with little modifications.

Rearing Protocol:

The *G. mellonella* larvae were first obtained from infested Apiculture in Abohamad El Sharkia, Egypt, classified as *G. mellonela* by Ahmed M. <u>Azazy</u> at the laboratory of the Physiology unit, Plant Protection Institute, Dokki where the colony was maintained and propagated according to Firacative *et al.*(2020).

Plastic containers (approximately 28 x 14 x 20 cm) were used for rearing the larvae. The container covers were replaced by paper and stretch ties to allow ventilation. Overall, the containers were well-ventilated and were able to contain young larvae (around 300 to 600 larvae per jar). Colonies were sporadically stressed by mold or bacterial growth. In this case, the affected larvae (presenting an increase in melanin production and/or slow movements) and the contents of the plastic containers were disposed of appropriately, with containers washed and sterilized before reuse.

The time taken for *G. mellonella* to complete its life cycle is affected by temperature. It can take 8–10 weeks at temperatures between 28 and 34 °C, but up to 13 weeks at room temperature (24 °C). The larvae feed and develop into pupae and then adults. After the mating takes place, the female moths lay eggs on the paper stripes. The deposited eggs on the paper strips could be collected daily or every 48 h and placed in a new rearing container with a proper amount of the prepared diet. The eggs hatch and the emerged larvae feed on the diet and develop through six larval instars before pupations. Larvae and pupae were reared and kept in incubators. An environmentally controlled rearing room is required. The controlled conditions of incubators are 26 °C and 60% relative humidity and kept in a relatively dark area where they would not be disturbed aside from feeding and larval collection periods. The last instar larvae could be collected to be used in producing

the nematodes in vivo.

Basic Approaches for Mass Rearing of Entomopathogenic Nematodes EPNs (Unit B): In This Section Methodology Were Clarified in Detail as follows:

Different EPNs species can be included in this study as indigenous species in Egypt like *Steinernema carpocapse*, *S. abbasi*, *S. riobrave*, *S. kushidai*, *S. glaseri*, and *Heterohabditis bacteriophora*. *H. indica*, *H. egyptii* and *H. taysearae*. However, experimental work in this study was carried out on *Steinernema carpocapse* (as an ambush predator which predates near the soil surface) and *Heterohabditis bacteriophora* (a forager) according to McMullen & Stock (2014).

The Rearing Process of EPNs Involves Four Major Steps: 1-Colony Assessment and Population Density Count:

The first step includes an assessment of EPNs colonies' population density which is done to determine the number of nematodes per microliter of solution. This process is conducted by (1) taking a specific volume of EPNs colony 75 μ l from the tissue flask using the micropipette and placing it on a small 60 mm petri dish that has had a grid scratched onto the bottom of it (taking a larger sample with a less concentrated colony and a smaller sample with a more concentrated colony).(2) The petri dish is then filled with deionized water until it fully covers the bottom and set under the microscope (10 x) or magnifying lens (3) counting the number of EPNs in the petri dish and this step was repeated three times to achieve a reliable population count to obtain the average number of nematodes per microliter then divide by the number of microliters used in the sample volume.

Infection was at a rate of 20 nematodes per host, to determine the total volume of nematodes to infect with, the total number of nematodes required per petri dish was divided by the number of nematodes per microliter which was previously determined, the answer will be the number of microliters required for an infection in each petri dish using the micropipette. The correct volume of nematodes was placed in each petri dish and approximately a half milliliter of deionized water per infection arena was added then the cover was added to the petri dish and was labeled with the nematode species and the infection date.

EPNs were stored at dark and warm or room temperature locations for one week. It can be simply covering the petri dishes with a cardboard box.

The number of infected petri dishes or infection arenas used is determined by whether you want to simply maintain population levels or increase population levels: If you wanted to maintain a colony population, 5 hosts per arena and infect three arenas per week, if you wanted to increase populations in preparation for application of the nematodes you could either increase the number of hosts per infection arena or / as well as increase the number of infection arenas created or both of them.

2-Host Infection:

The second step involves the infection of the Late instar of *G. mellonella* with a specific species of EPNs which was completed by applying a predetermined amount of EPNs (infection was at a rate of 20 nematodes per host), in the center of each petri dish which containing the wax worm hosts inside, a few drops of deionized water were placed in each petri dish to facilitate EPNs movement and for moisture.

To determine the infection rate, the number of nematodes per microliter which was previously determined in the population density count(X) and divided by one and then we multiply it by the number of nematodes desired (DN), we infect at a rate of 20 nematodes per host therefore with a petri dish with five hosts you multiplied by a hundred getting the total number of microliters necessary per petri dish (TN).

$$TN = \frac{1}{x} x DN$$

Where, **(TN)** the required microliters of nematode necessary per petri dish. **(X)** is the number of nematodes per microliter which was previously determined in the population density count.

(DN) the number of desired nematodes per petri dish (20 nematoda / host).

3-White Trap (White, 1927):

The third step the white trap or extraction takes place one week after the infection, the small petri dish 60 mm with infected hosts is placed in a larger petri dish 100 mm with water covering the bottom, this purpose is to attract the nematodes when they emerge from the infected cadaver into the pool of water. The lid of the smaller 60 mm petri dish was removed and the cover on the larger 100 mm petri dish was placed the labels were transferred onto the larger petri dish and marked with the date, the petri dishes were kept in a dark area warm or room temperature for an additional three to four weeks in the same place of the infection.

Before begging the white trap, the infected hosts were assessed to remove any of the wax worms if any of the hosts are still alive or any fungus develops or they look diseased, or not in the proper color. The infected *Steinernema carpocapse* should be a beige color while the infected *Heterohabditis bacteriophora* should be a dark brick red.

4-Harvest Reared EPNs:

In the fourth step, after three weeks in the white trap the larger petri dish full of water and EPNs is poured into a tissue flask and eluded with additional water the EPNs are then stored in a flask for a few weeks and up to two months before the process can begin again.

5-Storage of EPNs:

The tissue flasks containing EPN liquid suspensions can be stored in a cabinet or in cooler temperatures such as in a refrigerator or at a controlled room temperature of 4 - 15°C for a longer period (Chen and Glazer, 2005). An aerated environment in the tissue flasks was accomplished by using an aquarium air pump in an airstone stainless steel (they have smaller pore sizes increasing the rate at which the oxygen will dissolve into the water).

Also, an aqueous suspension can be used for both transport and application (Shapiro-Ilan et al. 2015). The storage temperature is $4 - 15^{\circ}$ C has produced survival times of 6–12 months for *Steinernema* spp. and 3–6 months for *Heterorhabditis* spp. (Hazir *et al.*, 2003).

RESULTS

1-Sales Prices and Costs Involved with The Rearing of *G. mellonella* and Entomopathogenic Nematoda (EPNs):

1.1 Fixed Assets:

Unit (A) *G. mellonella*, the insect host contains a number of machinery and utility in mass production with a total price of Unit (A) (111500 LE.). Unit (B) while (EPNs), with a total price of Unit (B) (11180 LE). Finally, the total price of Units (A+B) = 122680 LE (Table 1).

1.2 Working Capital:

1.2.1 Raw Materials (variable cost) (food and other)/ year.

The raw materials constituting the artificial media for Unit (A) for one year cost 166000 LE (Table 2) while the cost of EPNs packaging is 25000 LE with a total cost of 187000 LE. Data in Table 2 showed the prices of all raw materials in Egypt during 2022 purchased in large quantities to get the best price.

1.2.2 Fixed Capital (F.C.):

Fixed capital represented by machinery and utility is 122680 LE and furniture

cost 10000 LE with a total of 132680 LE.

1.2.3. Working Capital (W.C.):

Containing salaries/three months 60000 LE, rents/one year LE 36000, water and electricity / three months according to statistics 3000 LE (El Arnaouty, 2001), marketing costs / three months 5000 LE and other costs / three months 8000 LE with the working capital total price of 106000 LE (Table 3).

1.3.Total invested capital

Total invested capital = F.C + W.C. Total invested capital = 132680 + 106000 = 238680 LE.

1.4 Other fixed costs for a year:

Other fixed costs for a year including raw materials 187000 LE, rents 36000 LE, salaries 240000 LE, electricity & water 12000 LE, marketing costs 20000 LE, other costs 8000 LE, the total of operating expended 503000 LE (Table 4). The total number of skilled human resources is four. Two laboratory technicians are required whose salary will be 6000 LE/month for each one. Two laborers will be hired for 4000 LE /month for each one as indicated in Table 5. The total operation of *G. mellonella* rearing and EPNs production besides other equipment requires two rooms of 5x5 area. The rent is given as 36000 for the first year (Table 6).

1.5 Depreciation:

The depreciation of the year represented by machinery & utility 18402 LE and furniture 1000 LE, with total depreciation per day of 19402 LE (Table 7).

2. Estimation of the Production Costs of EPNs:

The price of one sachet equals 200 LE (Table 8). Year profit value is calculated as production for the year less other fixed costs giving 9497000 LE and the percentage of profit was recorded at 3978 %, while the capital recovery period was 99.7 % after the 1^{st} year from the fixed assets and costs.

Unit	Serial	Capital Cost	Price of unit	Number	Total price
	1	Incubator binder BD 56	30000 1		30000
	2	Air conditioner (2000 BTU)	15000	2	30000
	3	Refrigerator 18 Cu.Ft	12000	1	12000
	4	Freezing unit	12000	1	12000
	5	Angle iron shelving unit each 4 shelves	1000	6	6000
		$(3 \times 0.5 \times 3 \text{ m})$			
	6	3 units /room	1.40	100	16.000
	6	Rearing containers	140	120	16.800
		20 Containers / Angle iron shelving unit 28 x 14 x 20 cm; 250 Grams			
slla	7	Classical aspirator	100	2	200
lone	8	Sieve	50	1	100
t A nel	9				
Unit A Galleria mellonella	9	Wooden table (working area) 150/205/260x95 cm	1500	2	3000
aller	10	Table lamp	150	2	300
G	11	Kitchen spatula.	50	6	300
	12	Electronic balance.	200	2	400
	13	Large pan (5 Kg capacity)	200	2	400
		Total (A)		111500	
	14	100 mm Petri dish	10	60	600
	15	60 mm plastic Petri dish	5	60	300
	16	Filter paper (Whatman No. 1) package (100 paper) or similar absorbent paper.	50	10	500
	17	0-200 ml Micropipette variable Accumax (or syringe).	500	2	1000
	18	1 L tissue flask (or any container modified to allow for air flow).	150	10	1500
	19	10 X magnifying optical microscope (popular keen) microscope (hand lens).	3380	1	3880
	20	Forceps.	40	10	400
-	21	Nematode counting chamber.	800	1	800
Unit B (EPNs)	22	Absorbent material for lining the inoculation and harvest trays such as	20	20	400
	23	paper towels packages. Holding containers or buckets for holding harvested IJs.	100	10	1000
	24	0.5 Micron Stainless Steel air stone with micro bubble	80	10	800
		Total (B)			11180
		Capital cost total			122680

Table 1: Machinery and utility in mass production for one year.

	Materials			Quantity	Price of unit / IE	Total price
	Corn 120 containers x 1Kg x12 m	onth	Unit Kg	1440	20	28800
supplements:	Wheat 120 containers x 0.5Kg month	x12	Kg	720	20	14400
	Honey 120 containers x 0.25 Kg month	x12	Kg	360	25	9000
G.mellonella artificial Diet &	Powdered Milk 120 containers x 0.25 Kg month	x12	Kg	360	90	32400
	Glycerin 120 containers x 0.25 Kg month	x12	L	360	170	61200
	Yeast (inactive) 120 containers x 0.25 Kg month	x12	Kg	360	45	16200
						166000
	Plastic packages		Packag	50000	0.5	25000
			Sachets			
			187000			

Table 2: Raw materials r	represented by feeding	materials and others	(Variable cost) /
year.			

Table 3: Total invested capital.

Serial	Serial Fixed capital/year Cost/ LE		Total cost/ LE
1	1 Machinery and utility 122680		
2	2 Furniture 10000		
Total of fixed capital		132680	
Serial	Working capital	Cost/ LE	Total cost/LE
1	Rents/year	36000	
2 Salaries / three months 60000		60000	
3 Electricity & water / three months 3000			
4 Marketing cost / three months 5000			
5 Other costs/ three months 2000			
Total working	106000		
Total invested	238680		

Table 4 : Other fixed costs for year.

Serial	Operating expended	Cost /LE
1	Raw materials	187000
2	Salaries	240000
3	Rents	36000
4	Electricity & water	12000
5	Marketing costs	20000
6	Other costs	8000
	Total of operating expended	503000

Table 5: Human resource requirement (Salaries).

Description	Amount
Laboratory technicians' salary (2) / month	12000
Labor (2) / month	8000
Total / month	20000

Table 6: Space requirement.

Description	Amount	
Rent of 2 room (5x5 ft.)	36000/year	

Table 7: Deprecation Value.

Serial	Materials	Deprecation % of year	Deprecation Value/ year
1	Machinery & utility	0.15	18402
2	Furniture	0.1	1000
3 Total deprecation value / day		19	402

Table 8: Total costs of EPNs Sachest.

Producible	productive potential /year			Total salaries (LE)
EPNs sachets	Quantity	Unit	Salary price / LE	
Н.	40000	Sachet (50 million)	200	8000000
bacteriophora				
S.	10000	Sachet (50 million)	200	2000000
carpocapsae				
Total	1000000			

DISCUSSION

Entomopathogenic nematodes belonging to the genera Steinernema and Heterorhabditis are effective against more than two hundred soil insect pest species and are employed as environmentally friendly biopesticides to control key pests in high-value agricultural crops (Labaude & Griffin, 2018). Their successful application in developed countries has proved their effectiveness. The production of EPN for large-scale commercial applications has been restricted to developed countries because of the high capital cost of setting up liquid fermentation, and the high running costs of such units. EPNs can be mass-produced by in vivo or in vitro approaches for commercial production or lab tests (Shapiro-Ilan *et al.*, 2015). If EPNs are to be commercialized in developing countries, then this will probably be achieved using in vivo culturing in insect larvae. Supplying indigenous isolates offers the promise of producing locally adapted strains, rather than one size fits all when depending on purchasing the online strains from Europe and USA.

The present feasibility study of G. mellonella rearing and production of EPNs is carried out to study the idea of this project to find out whether it is actually a profitable project before implementing it on the ground, and based on the final result of this study, a decision is obtained about whether the project is economically feasible, or otherwise, rearing G. mellonella larvae is often less costly compared to other nematode's natural hosts. Because they have short life cycles and readily available and affordable diet components, a steady and prompt supply of larvae w ill be available during the production process (Van Zyl & Malan, 2015). Also, their use either in academia or industry is not restricted by legal or ethical considerations (Piatek et al., 2021). These methods have proven to be successful in the laboratory at the Physiology unit, Plant Protection Institute, Dokki. Each rearing container needs about 0.25 Kg which is renewed three times during the month so each rearing container consumed about 1 kg per month, so 120 rearing containers consumed about 1440 Kg per year which cost 166000 LE per year as indicated in table (4) for all the constituents of the culture medium. Diet component prices rely on factors such as geographic location, market type (such as feed or food), and quantity sold. In comparison to Western nations, prices were relatively low in nations with low operational costs. Due to the higher necessary quality for food production and the smaller quantities sold, prices for products used for food were often higher than prices for feed. Prices have fallen over time, which may be attributed to increased competition as well as stable demand. Since the market for insects as feed and food has increased by 10 to 25 percent annually, for instance, the market conditions were different in 2016 than they were in 2010 (Van Huis, & Oonincx, 2017).

This study identified the production of EPNs that avoids the extensive capital and technical requirements of other methods. It also allows for the use of unskilled and semi-skilled labour as it is not technically complex. The expected production power is high starting with 60 Petri dishes containing 5 hosts per arena at least, the number of infected larvae will be 300 larvae, the infection can be repeated during the year each month after excluding 3 months to start the colony of G. mellonella and the first infection so the process of infection can be repeated 9 times to obtain 2700 infected larvae at least. From each infected host more *H. bacteriophora* juveniles around a million were harvested while hosts infected by S. carpocapsae gave numbers which are less 4 folds than H. bacteriophora (Tourtois et al., 2017). In vivo production of S. carpocapsae and H. bacteriophora on G. mellonella is approximately 200,000 IJs per host (Dutky et al., 1964; Selvan et al., 1993 and Flanders et al., 1996). EPNs were applied in an aqueous solution. Also, EPNs can be formulated and applied as insect cadavers which showed elevated efficacy for pest control when EPNs pre-infected live insect hosts were released against pests living in cryptic habitats (Gumus et al., 2015). It found that cost was about 10 \$ (200 LE) per 50 million IJs as indicated in Table 8, 50 million IJs cover 2,000 - 2,500 sq. ft. The estimated retail price for this production system is considerably lower than the cost of current products on the market, for example, Bug company sales 50 Million Live Beneficial Nematodes - HB (Heterohabditis bacteriophora) for 34.50 \$ (https://www.amazon.com/Bug-Sales), also the price of Steinernema carpocapsae Nematodes 10 million (Product ID: SC10) is 29.95 \$ (https://www.buglogical).

Most of the nematode-based products recently available include formulations of various strains (Kaya *et al.*, 2006). Nematode products come in pack quantities at concentrations ranging from 5 million to 500 million (Floyd *et al.*, 2012). Nematode products are generally more expensive than their chemical counterparts (Wright *et al.*, 2005) due to the high cost of production, formulation and transportation. Controlled environmental conditions (temperature & aeration) during production and transportation add to the high cost of these products. The price of products is variable from about US\$ 15 to US\$ 160 per sales unit (Dolinski, *et al.*, 2012).

The present study percentage of profit (3978 %) and the capital recovery period (99.7 %) not only made EPNs control of key pests in high-value agricultural crops accessible but also, unexpectedly, as with many pest control tactics, low-value field crops may support the cost of pest management with EPNs according to costs and prices in Egypt at the time of this study. Nevertheless, the commercialization of EPNs was the result of long-term, broad-based, publically supported incorporations of scientists and growers to encourage the development and use of biological controls. Continuing interest by these groups in the dynamics of classical biological control of insect pests supports the likelihood of future commercialization of this nematode.

Conclusion

There wasn't much financial information available on *G. mellonella* rearing and production of EPNs in vivo prior to the publishing of this study. Data concerning the financial feasibility of insect farming is vital for policymakers, investors, banks and other stakeholders to assess credit risk, and formulate policies and regulations for this emerging sector. This study gives insights into the smaller SME's where a lot of labour is involved which can compete with the high-tech industrialized businesses. As stated before a cooperative model between insect rearing/reproduction farms is widely addressed. As different smaller farms cooperate to reach higher volumes and more consistent quality and quantity of their product. This is an interesting topic for further research in order to increase certainty and scale of supply which will trigger innovation and adoption further down the value chain.

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REFERENCES

- El-Arnaouty, S. A. (2001). Technical improvements in mass rearing of Ephestia kuehniella Zeller (Lep.: Pyralidae) as a substitution prey for a cost-effective production of Chrysoperla carnea Steph. *Integrated Pest Management, Proc. Of the 1st* Congress, Cairo Univ., Fac. Agric (pp. 22-23).
- Chen, S.; Glazer, I. (2005). A novel method for long-term storage of the entomopathogenic nematode Steinernema feltiae at room temperature. *Biological Control*, *32*(1), 104-110.
- Dolinski, C.; Choo, H.; Y., Duncan, L. (2012). Grower acceptance of entomopathogenic nematodes: case studies on three continents. *Journal of Nematology*, 44(2), 226.
- Dutky, S.R.; Thompson, J.V.; Cantwell, G.E. (1964). A Technique for the Mass Propagation of the DD-136 Nematode. *Journal of Insect Pathology*, 6: 417–422.
- Firacative, C.; Khan, A.; Duan, S.; Ferreira-Paim, K.; Leemon, D.; Meyer, W. (2020). Rearing and maintenance of Galleria mellonella and its application to study fungal virulence. *Journal of Fungi*, 6(3), 130.
- Flanders, K.L.; Miller, J.M.; Shields, E.J. (1996). In Vivo Production of Heterorhabditis bacteriophora "Oswego" (Rhabditida: Heterorhabditidae), a Potential Biological Control Agent for Soil-Inhabiting Insects in Temperate Regions. *Journal of Economic Entomology*, 89: 373–380.
- Floyd, L. I.; Singh, S.; Holmes, L. D. (2012). Mass production of the beneficial nematode Heterorhabditis bacteriophora and its bacterial symbiont Photorhabdus luminescens. *Indian Journal of Microbiology*, 52(3), 316-324
- Friedman, M. J. (1990). Commercial production and development. *Entomopathogenic nematodes in biological control*, 153-172.
- Hazir, S.; Kaya, H. K.; Stock, S. P.; & Keskin, N. (2003). Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish journal of Biology*, 27(4), 181-202.
- Ites, S., Smetana, S., Toepfl, S., & Heinz, V. (2020). Modularity of insect production and processing as a path to efficient and sustainable food waste treatment. *Journal of*

Cleaner Production, 248. doi: 10.1016/j.jclepro.2019.119248

- Kaya, H. K.; Aguillera, M. M.; Alumai, A.; Choo, H. Y.; De la Torre, M.; Fodor, A., ... & Ehlers, R. U. (2006). Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological control*, 38(1), 134-155.
- Kusuma, C. M. (2021). Role of cottage industries in socio-economic development of women workers-A study across Bangalore Rural District. *PalArch's Journal of Archaeology of Egypt/Egyptology*, 18(09), 353-362.
- Labaude, S.; Griffin, C. T. (2018). Transmission success of entomopathogenic nematodes used in pest control. *Insects*, 9(2), 72.
- Lacey, L. A. (Ed.). (2016). Microbial control of insect and mite pests: from theory to practice. Academic Press.
- Madau, F. A.; Arru, B.; Furesi, R.; Pulina, P. (2020). Insect farming for feed and food production from a circular business model perspective. *Sustainability*, *12*(13), 5418.
- McMullen II, J. G.; Stock, S. P. (2014). In vivo and in vitro rearing of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae). *Journal of Visualized Experiments*, (91), e52096.
- Morales-Ramos, J. A.; Rojas, M. G.; Shapiro-Ilan, D. I. (Eds.). (2013). Mass production of beneficial organisms: invertebrates and entomopathogens. Academic Press.
- Nagesh, M.; Askary, T. H.; Balachander Manohar, B. M.; Arakalagud, S. N.; Rajan, R. (2017). Strategies for making entomopathogenic nematodes cost-effective biocontrol agents. In *Biocontrol agents: entomopathogenic and slug parasitic nematodes*, (pp. 596-619). Wallingford UK: CABI.
- Piatek, M.; Sheehan, G.; Kavanagh, K. (2021). Galleria mellonella: The Versatile Host for Drug Discovery, In Vivo Toxicity Testing and Characterising Host-Pathogen Interactions. *Antibiotics*, 10(12), 1545.
- Saleh, M. M.; Metwally, H.; Abonaem, M. (2020). Commercialization of biopesticides based on entomopathogenic nematodes. In *Cottage Industry of Biocontrol Agents* and Their Applications, (pp. 253-275). Springer, Cham.
- Selvan, S.; Campbell, J.F.; Gaugler, R.; 1993. Density-Dependent Effects on Entomopathogenic Nematodes (Heterorhabditidae and Steinernematidae) within an Insect Host. *Journal of Invertebrate Pathology*, 62, 278–284.
- Shapiro-Ilan, D.; Dolinski, C. (2015). Entomopathogenic nematode application technology. *Nematode pathogenesis of insects and other pests*, 231-254.
- Thakur, N.; Kaur, S.; Tomar, P.; Thakur, S.; Yadav, A. N. (2020). Microbial biopesticides: current status and advancement for sustainable agriculture and environment. *In New and future developments in microbial biotechnology and bioengineering*, (pp. 243-282). Elsevier
- Tourtois, J.; Ali, J. G.; Grieshop, M. J. (2017). Susceptibility of wounded and intact black soldier fly Hermetia illucens (L.) (Diptera: Stratiomyidae) to entomopathogenic nematodes. Journal of Invertebrate Pathology, 150: 121–129.
- Van Huis, A.; Oonincx, D. G. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, *37*(5), 1-14.
- Van Zyl, C.; Malan, A. P. (2015). Cost-effective culturing of Galleria mellonella and Tenebrio molitor and entomopathogenic nematode production in various hosts. *African Entomology*, 23(2), 361-375.
- Vashisth, S.; Chandel, Y. S.; Sharma, P. K. (2013). Entomopathogenic nematodes-a review. *Agricultural Reviews*, 34(3).
- White, G.F., 1927. A Method for Obtaining Infective Nematode Larvae from Cultures.

Science, 66, 302–303.

Zhang, X. (2005). Financial viability analysis and capital structure optimization in privatized public infrastructure projects. *Journal of Construction Engineering and Management*, 131(6), 656-668.

https://www.buglogical.com/beneficial-nematodes/steinernema-carpocapsae-nematodes/ https://www.amazon.com/Bug-Sales-Million-Beneficial-Nematodes/dp/B012TYT37K