PRODUCTION OF AN ENTOMOPATHOGENIC NEMATODE, *Acrobeloides longiuterus* USING ARTIFICIAL SOLID MEDIA

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Introduction

Acrobeloides longiuterus (Nematoda: Cephalobidae) is a free-living nematode [not a true member in the entomopathogenic genera (Steinernema and Heterorohabditis)] isolated from soils of Northern Sri Lanka using an insectbaiting technique. The propensity of this nematode to kill insect pests has been tested with many agriculturally important pests under in-vitro and field conditions [1], [2] and it shows the potential to be used as a biological control agent in future. However a bio-control agent is required to be used in large scale production and readily available in required quantity with low cost of production throughout the year for the entrepreneurs and farmers [3]. Therefore, a study was carried out with an objective; to evaluate the production efficiency of A. longiuterus on different solid media and its quality on insect-pathogenicity.

Materials and Methods

In-vitro culturing of *Acrobeloides longiuterus* Culturing of A. longiuterus medium [4] and newly developed a medium. All the ingredients shown in the Table 1 were added into the conical flask and contents were mixed with 1 L of distilled water using a magnetic starrier at a temperature of 75 oC for five minutes. Conical flasks plugged with cotton were sterilized using an autoclave at 121 oC, 1.054 kg/cm2 for 20 minutes. The culture medium was transferred into a 90 mm diameter petri-dish (15mL/petri-dish) under aseptic conditions. Subsequently, symbiotic bacteria isolated from nematode was inoculated into each medium and incubated for four days. On the grownup bacterial culture, a 6 mm hole was made in the middle using a cork borer. Subsequently 100 Infective Juveniles (IJs) were introduced into the hole. This was replicated four times. Continued observation and monitoring were conducted until the nematode development was visible. IJs were collected using White trap techniques following 15 days inoculation. Pathogenicity of *Acrobeloides longiuterus* against Tribolium castaneum A moisture chamber assay was used to test the quality of A. longiuterus using invitro production methods as described by Kaya and Stock [5]. T. castaneum larvae and pupae were exposed to the different concentrations. An experiment was arranged with four different concentrations of 50, 100, 150 and 200 IJs/mL/petri-dish and distilled water was used as the control. Moisture chambers containing 10 larvae and 10 pupae were separately used to apply the above concentrations and, were replicated four times. Pupal and larval mortality were recorded on 2nd and3rd days after inoculation, respectively. Statistical analysis Data were analysed using one-way ANOVA and mean separation was carried out according to the Fisher LSD method at the 95 % of the confidence interval. Probit analysis was used to calculate the LC50 and LC90 values using the software of Minitab 17.

Results and Discussion

Acrobeloides longiuterus production on different media under in-vitro conditions In-vitro production of A. longiuterus media composition are given in the Table 1. Highest IJs were produced in the medium IV (Nutrient agar, Soy flour, sun oil and glycerol), which was found to be 2.01 X 106 IJs/15mL, followed by 8.98 X 105 , 5.5X 105 and 3.43 X 105 IJs/15mL respectively. This yield of IJs comparably high with the results obtained by the ElSadawy [4]. He reported that, Steinernema carpocapsae, S. scapterisci, S. riobrave, S. carpocapsae, S. abbasi, S. glaseri and S. spp, 5, 8, 8.5, 5.5, 6, 4 and 3 compositions of the media and cultured

nematodes species are different. Quality testing of *Acrobeloides longiuterus* against Tribolium castaneum IJs production in different in-vitro media was tested against T. castaneum. Mortality of the larval and pupal stages at different concentrations is given in the Table 2. Mortality of larvae at all concentrations of A. longiuterus IJs/mL/petridish was significantly different from the untreated control. Highest mortality of larvae and pupae (92.5%) was recorded at the concentration of 200 IJs/mL/petridish. LC50 and LC90 of the larvae were calculated as 48.55 and 210.42 IJs/mL/petridish, respectively. All the concentrations of A. longiuterus IJs against pupal mortality was significantly different from the control. Pupal mortality rates of 65, 75 and 90 % were recorded at the concentrations of 50, 100, 150 IJs/mL/petri-dish, respectively. LC50 and LC90 of the pupae were calculated as 32. 94 and 173.97 IJs/mL/petri-dish, respectively. Pupal mortality was caused by the IJs produced from the T. castaneum larvae and yielded a LC50 of 10.64 IJs/mL/petri-dish.

Table 1. Production of infective juveniles' of *Acrobeloides longiuterus* under *in-vitro* conditions using different media

Components	Media			
	I	II	III	IV
Nutrient Broth (g)	16	16	-	-
Nutrient agar (g)	-	-	32	32
Bacteriological Agar (g)	12	12	-	-
Yeast extract (g)	6	6	-	-
Soy flour (g)	7	7	7	7
Sun flower oil (mL)	5	5	5	5
Glycerol (mL)	5	5	5	5
NaCl (g)	0.5	-	0.5	-
$CaCl_2$ (g)	0.21	-	0.21	-
KH_2PO_4 (g)	2	-	2	-
Distilled water (L)	1	1	1	1
IJs/15 mL of medium* (10^6)	0.55 ± 0.12^{bc}	0.34 ± 0.05^{c}	0.90 ± 0.27^{b}	2.01 ± 0.43^{a}
IJs/mL of medium (10^3)	36.67	22.88	59.87	134.21

** Each value represents the mean value from four replicates. . *Values having the same letter are not significantly different according to the Fisher LSD at 95 % confidence interval*

Table 2. Mean mortality of *Tribolium castaneum* larvae and pupae by *Acrobeloides longiuterus* produced from *in- vitro* media

Concentration IJs/mL/petri-dish	Mean Mortality*	
	Larva	Pupa
0	[d]0.25±0.500	[d]0.5±0.577
50	[c]5.25±0.500	[c]6.5±0.577
100	[b]7.25±0.500	[b]7.5±0.577
150	[b]8.00±0.816	[a]9.00±000
200	[a]9.25±0.500	[a]9.25 ±0.5
LC_{50}	48.55	32.94
LC_{90}	210.42	173.97

** Each value represents the mean value from four replicates. Values having the same letter in a column were not significantly different according to the Fisher LSD at 95 % confidence interval*

Conclusions and recommendations

Acrobeloides longiuterus successfully cultured in a developed nutrient agar based medium (2 million IJs/15 mL) and without losing their insect-killing (more than 92 %) quality. Therefore, the nutrient agar based medium is better for this nematode multiplication in a small scale level and it can be used for the pest management programs. However, the testing in different formulations in fields need to be conducted before the commercialization.