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MASS MULTIPLICATION OF ENTOMOPATHOGENIC NEMATODES IN ARTIFICIAL MEDIUM

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Mass multiplication of entomopathogenic nematodes, *Heterorhabditis becteriophora* and *Steinernema carpocapsae* were done on different artificial media used animal and plant protein or in combination of both. The media were prepared in different composition and filled with 10 sponges of polyether polyurethane and EPNs were inoculated, incubated at 25±1°C for 30 days. The highest number of infective juveniles of *H. becteriophora* (11.65×10^5) was harvested from Wout's medium followed by modified Wout's medium (7.21×10^5), similarly in *S. carpocapsae* infective juveniles were harvested (13.65×10^5) from modified egg yolk medium and 9.37×10^5 infective juveniles from Wout's medium.

Keywords : Artificial media; *Heterorhabditis becteriophora; Steinernema carpocapsae.*

The use of alternatives of chemical control, in insect pest managements, is increasing with increased awareness of its ill effects on environment and residue problems. The use of entomopathogenic nematodes is being explored as a component of integrated management of persistent insects in sustainable agriculture. The success of their integration depends much on the ability to mass multiply EPN for field application. In the present studies, an attempt has therefore, been made to evaluate the mass multiplication efficiency in different media.

The artificial media were prepared from different animal and plant protein ingredient according to given composition with polyether polyurethane sponges (1.5 cm^3). The polyether polyurethane sponges were used as the substratum of entomopathogenic nematode multiplication. The prescribed quantity of medium was mixed with sponges chips till the latter got soaked in the medium. The 10 (1 sponges containing 1 g media) sponge chips were filled in 250 ml conical flask and plugged with cotton. The flasks were autoclaved for 20 minute at 120°C and allowed cooling at room temperature. The nematodes were inoculated in the flask @ 500 IJs/flask under aseptic condition and treatments were replicated four times. The sealed flasks were incubated at 25°C for 30 days. The nematode yield from each medium harvested was expressed in terms of number of IJs/250 ml flask.

The data on yield, depicted in the Table 1 revealed that the maximum number of IJs of *Heterorhabditis bacteriophora* (11.65×10^5) was harvested in the Wout's medium followed by modified Wout's medium (7.21×10^5), egg yolk medium II (6.34×10^5), egg yolk I (4.55×10^5) and modified wheat flour medium (3.18×10^5).

Similar studies in this regards were made by earlier workers[1-3] who reported that maximum yield (30.58×10^5) of *Steinernema* sp. (SSL2) PDBCEN 13.21 IJs was recorded from Wout's medium followed by dog biscuit + peptone + beef extract (24.5×10^5), dog biscuit + beef extract (18.40×10^5), dog biscuit + peptone (12.12×10^5) and dog biscuit + bacterial culture (10.14×10^5). The mass production of native *Steinernema* sp. was observed by using 21 animal and plant protein media. Maximum production of EPNs was recorded in hen egg yolk medium which was economically better than universally used dog food biscuit agar. It was also reported that production of the entomopathogenic nematode was poor in plant protein in comparison to animal based media. The different plant and animal protein media were used for production of 3 indigenous isolates of *Steinernema carpocapsae*, two isolates of *S. bicornutum* and one of *Heterorhabditis indica,* and recorded that Wout's medium, modified egg yolk, soyflour + cholesterol and modified dog biscuit, yielded highest number of infective juveniles of two isolates of *S. carpocapsae*, one isolate of *S. bicornutum* (PDBC EN 2.1) and one of *H. indica* (PDBC EN 6.71). Maximum yield of IJs of *S. carpocapsae* (PDBC EN 6.11 and 6.61) was observed on modified dog biscuit.

References

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2. Hussaini S S, Singh SP, Parthasarathy R and Shakeeh

Ingredients of media

Wout's medium :

Nutrient broth	– 0.88 g
Yeast extract	– 0.32 g
Groundnut oil	– 10.40 g
Soy flour	– 14.40 g
Distilled water	– 60 ml

Modified Wout's medium :

Nutrient broth	– 0.44 g
Yeast extract	– 0.16 g
Distilled water	–27 ml

Wheat flour medium :

Wheat flour	– 15.00 g
Kabuligram flour	– 5.00 g
Beef extract	– 5.00 g
Yeast extract	– 6.00 g
Agar	– 1.00 g
Coconut oil	– 6.00 g
Distilled water	– 60 ml

Wheat germ medium I :

Wheat germ	– 5.00 g
Yeast extract	– 1.50 g
Agar	– 0.50 g
Distilled water	– 10 ml

Wheat germ medium II :

Wheat germ	– 10.00 g
Yeast extract	– 1.50 g
Agar	– 1.00 g
Distilled water	– 60 ml

Modified wheat-flour medium :

Wheat flour	– 15.00 g
Soy flour	– 5.00 g
Beef extract	– 5.00 g
Yeast extract	– 1.00 g
Groundnut oil	– 10.00 g
Distilled water	– 60 ml

Egg yolk medium I :

Solid egg yolk	– 7.00 g
Yeast extract	– 2.00 g
Sodium chloride	– 0.80 g
Oil	–15.00 g
Distilled water	– 60 ml

Egg yolk medium II :

Solid egg yolk	– 10.00 g
Yeast extract	– 5.00 g
Sodium chloride	– 0.80 g
Oil	–15.00 g
Distilled water	– 60 ml

Wheat-bran medium I :

Wheat bran	– 5.00 g
Yeast extract	– 1.50 g
Agar	– 0.50 g
Distilled water	– 60 ml

Wheat-bran medium II :

Wheat bran	– 10.00 g
Yeast extract	– 2.00 g
Agar	– 1.00 g
Distilled water	– 50 ml

Modified egg yolk medium :

Egg yolk	– 7.00 g
Soy flour	– 20.00 g
Yeast extract	– 2.00 g
Sodium chloride	– 0.80 g
Oil	–15.00 g
Distilled water	–60 ml

Dog biscuit medium :

Dog biscuit	– 15.00 g
Yeast extract	– 1.00 g
Peptone	– 3.00 g
Agar	– 2.00 g
Oil	–10.00 g
Distilled water	– 60 ml

Modified dog biscuit medium :

Dog biscuit	– 20.00 g
Peptone	– 0.50 g
Yeast extract	– 1.00 g
Beef extract	– 5.00 g
Oil	–7.00 g
Distilled water	– 100 ml

Wheat-bran medium III :

Wheat bran	– 7.00 g
Yeast extract	– 0.50 g
Beef extract	– 1.00 g
Agar	– 1.00 g
Distilled water	– 60 ml

Wheat-bran medium IV :

Wheat bran	– 15.00 g
Yeast extract	– 2.00 g
Beef extract	– 1.50 g
Distilled water	– 60 ml

Table 1. Mass multiplication (*in vitro*) of entomopathogenic nematodes (in lakh).

S.No.	Media	*Heterorhabditis bacteriophora*	*Steinernema carpocapsae*
1.	Wout's medium	11.65 ± 2.04	9.37 ± 1.76
2.	Modified Wout's medium	7.21 ± 1.61	6.54 ± 1.20
3.	Wheat flour medium	0.00	0.00
4.	Modified wheat flour medium	3.18 ± 0.85	0.69 ± 0.19
5.	Egg yolk medium I	4.55 ± 0.95	1.39 ± 0.65
6.	Egg yolk medium II	6.34 ± 1.10	3.86 ± 0.92
7.	Modified egg yolk medium	0.00	13.65 ± 2.34
8.	Dog biscuit medium	0.00	0.00
9.	Modified dog biscuit medium	0.00	7.26 ± 1.70
10.	Wheat germ medium I	0.00	2.19 ± 0.07
11.	Wheat germ medium II	0.00	1.76 ± 0.19
12.	Wheat bran medium I	0.00	2.48 ± 0.57
13.	Wheat bran medium II	0.00	0.00
14.	Wheat bran medium III	0.00	0.00
15.	Wheat bran medium IV	0.00	0.00

Mean of four replications

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