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Survival and infectivity of entomopathogenic nematode *Oscheius rugaoensis* in different formulations against wax moth, *Galleria mellonella*

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Abstract
Entomopathogenic nematode, *Oscheius rugaoensis* were formulated in six different formulations such as talc, sawdust, alginate gel, water dispersible granule, compost: charcoal powder mixture and water. All formulations tested *in vitro* at two temperatures (5 ^{0}C and 30 ^{0}C) to evaluate their storage and infectivity against wax moth, *Galleria mellonella.* Sawdust and Alginate gel formulations were enhanced highest survival of infective juveniles of *Oscheius rugaoensis* than the other formulations. Per cent survival of *Oscheius rugaoensis* infective juveniles (IJs) was 98.30% in sawdust and 97.30% in alginate gel up to 6th week of storage at 5 ^{0}C, whereas per cent survival was less (95.50% and 93.76 % respectively) at 30 ^{0}C up to 6th week of storage. Larval mortality of *G. mellonella* by *Oscheius rugaoensis was* 47.66%, 67.33%, and 81.66% at 24 h, 48 h and 72 h respectively at 5 ^{0}C whereas larval mortality was 43.66%, 64.66% and 78.00% at 24 h, 48 h and 72 h respectively at 30 ^{0}C.

Keywords: entomopathogenic nematodes, survival, infectivity, formulation, storage temperature

1. Introduction
Entomopathogenic nematodes (EPNs), *Steinernema*, *Heterorhabditis* and *Oscheius* in the family Steinernematidae, Heterorhabditidae and Rhabditidae respectively of the order Rhabditida are obligate parasites of insect pests and distributed in natural and agricultural soils. EPNs are considered as one of the most significant non-chemical alternatives to insect pest control due to their high reproductive potential, ease of mass production and their harmlessness to animals, humans and plants. Nematode formulation is the most important aspect in the commercialization of nematode as a biocontrol agent. After mass production, entomopathogenic nematodes are formulated for ease of use, transport and field application. High oxygen and moisture requirement of nematodes, microbial contamination, sensitivity to temperature and behaviour of infective juveniles in order to maintain viability and storage stability, limit the choice of the formulation method and ingredients. The infective stage nematodes need to be formulated into solid or semi liquid substrates soon after they are mass cultured which guarantee survival for a period necessary to market the nematode product.
Hence the present study was carried out to formulate *Oscheius rugaoensis* in different formulations and test their survival and efficacy against larvae of wax moth (*Galleria mellonella*).

2. Materials and Methods
2.1 Nematode culture
Indigenous culture of *Oscheius rugaoensis* was maintained in the lab on *G. mellonella* larva. Infective juveniles were collected from infected cadavers by using white trap method [1].

2.2 Formulations of *Oscheius rugaoensis*
2.2.1 Talc formulation
Talc powder (250 g) was added to 25 ml of distilled water in a 500 ml beaker and mixed thoroughly. Fifty ml of freshly harvested IJs of *Oscheius rugaoensis* (2000 IJs/ ml) were added in the above moisten talc and then the contents were thoroughly mixed till the nematode suspension spread over evenly into the talc.

2.2.2 Sawdust formulation

The sawdust material was grinded separately to get fine dust with the help of mixer and grinder and sieved with fine mesh and then sterilized under sunlight for 1 hr. Two hundred grams of sawdust were moistened by adding 50 ml of distilled water separately. IJs suspension of 50 ml (2000 IJs/ ml) were added evenly and mixed them gently till nematodes spread over into the saw dust.

2.2.3 Alginate gel

A solution of gel matrix was prepared by dissolving 2 g of sodium alginate in 100 ml of water and blended for 4-5 minutes. Drops of this solution when placed into a 100 mM solution of $CaCl_2$. $2H_2O$ (The complexing solution) formed discrete capsule of calcium alginate. Fifty milliliter of nematode suspension (2000 IJs/ ml) were placed into the solution of Sodium alginate which is water-insoluble, gelatinous, cream coloured substance and then dripped into the complexing solution which was continuously stirred. Capsules were allowed to complex for 20-30 minutes and then separated from the complexing solution by sieving, rinsed in deionised water and stored at a temperature of 5^0C and 30^0C for further survival and infectivity observations. The actual number of nematodes was determined by dissolving five capsules in 9.5ml of 0.5M sodium citrate containing 0.1% Triton X-100.The capsules were stirred with magnetic spin bar until dissolution (about 30 minutes), and the nematode in 1 ml of suspension were counted using a Hawksley counting dish.

2.2.4 Water dispersible granule (WDG)

Clay, aloe gel and starch were mixed at the ratio of 1:1:1. IJs suspension of 50 ml (2000 IJs/ ml) were added evenly and mixed them gently till nematodes spread over into the above mixture. Granules of 10-20 mm diameter were prepared and packed in polythene envelope.

2.2.5 Compost and charcoal powder mixture

The formulation in which vermicompost and charcoal powder were mixed at the ratio of 1:1. IJs suspension of 50 ml (2000 IJs/ ml) were added evenly and mixed them gently till nematodes spread over into the above mixture.

2.2.6 Control (Water)

Freshly harvested infective juveniles were washed twice in distilled water and 50 ml (2000 IJs/ ml) of suspension was stored in 250 ml conical flask. Flasks were closed with non absorbent cotton.

The prepared formulations were packed in polythene envelope and stored at a temperature of 5^0C and 30^0C for further survival and infectivity observation.

2.3 Bioassay study

2.3.1 Survival of infective juveniles of *Oscheius rugaoensis*

Survival of infective juveniles in different formulations were evaluated by weekly interval up to 6 weeks at 5^0C and 30^0C temperature, by diluting 1.00 g of formulated IJs in 5 ml distilled water from each and the per cent IJs survival was counted and the per cent mean data of survived IJs was recorded. Four replicates for each treatment were done. Data obtained in a per cent survival of IJs were transformed to arcsine for statistical analysis. Data were statistically analyzed using two factorial completely randomized block design.

2.3.2 Infectivity of *Oscheius rugaoensis* against *Galleria mellonella*

Soil bioassay

The experiment was conducted in 250 ml capacity beaker. Two fifty grams of sterilized soil were kept in each beaker and 15% moisture was maintained. Five grams of *O. rugaoensis* formulations each with 5 replications were tested against 10 larvae of the greater wax moth, *G. mellonella*. Observations on mortality were done at 24 h intervals for three days. The data from percent larval mortality induced by *O. rugaoensis* were transformed to arcsine for statistical analysis. Data were statistically analyzed using Two factorial Completely Randomized Block Design.

3. Results and Discussion

3.1 Survival of infective juveniles of *Oscheius rugaoensis* in different formulations

Irrespective of storage time, the formulation (T) of Sawdust and Alginate gel were found to be significantly effective in survival of *O. rugaoensis* both at 5^0C and 30^0C when compared with control (water). Between the two formulations, survival per cent was found to be more in Sawdust than Alginate gel. Similarly, irrespective of formulation (T), the storage period (t) was also significantly effective for the survival of *O. rugaoensis.* Following the significant interaction of formulation and storage time (Txt), up to 3rd week of storage both at 5^0C and 30^0C, *O. rugaoensis* survival was 100% in sawdust and Alginate gel formulation and it was at par with control (water). After 4th week storage at 5^0C it was observed that, survival of *O. rugaoensis* in Alginate gel formulation, though significantly higher as compared to control, yet it was lower as compared to Sawdust formulation (97.20%). During the 6th week of storage, survival % of *O. rugaoensis* at 5^0C was significantly higher in Sawdust as compared to Alginate gel (93.00%) and control (88.00%) (Table. 1). Similarly at 30^0C, survival % was significantly higher in sawdust as compared to Alginate gel (80.60 %) and control (81.20 %) (Table. 2).

Table 1: Per cent survival of infective juveniles of *Oscheius rugaoensis* in different formulations stored at 5 ^{0}C (Mean of five replications)

Formulations	Survival (%)						Mean
	1st week	2nd week	3rd week	4th week	5th week	6th week	
Talc	100 (89.78)	100 (89.78)	100 (89.78)	85.20 (67.49)	81.60 (64.66)	77.60 (61.78)	90.70 (77.21)
Sawdust	100 (89.78)	100 (89.78)	100 (89.78)	99.40 (87.09)	97.60 (83.12)	93.20 (76.44)	98.30 (86.00)
Alginate gel	100 (89.78)	100 (89.78)	100 (89.78)	97.40 (84.07)	93.60 (75.83)	93.00 (74.87)	97.30 (84.02)
Water dispersible granule (WDG)	92.80 (76.13)	82.60 (65.46)	79.80 (63.36)	76.00 (60.73)	74.00 (59.43)	67.00 (55.00)	78.70 (63.35)
Compost : Charcoal powder mixture (1:1)	100 (89.78)	98.80 (86.14)	97.00 (83.60)	89.80 (73.62)	84.80 (67.35)	82.80 (65.91)	92.20 (77.73)
Control (water)	100 (89.78)	100 (89.78)	100 (89.78)	95.60 (79.58)	90.20 (73.97)	88.00 (70.17)	95.63 (82.18)
Mean	98.80 (87.51)	96.90 (85.12)	96.13 (84.35)	90.56 (75.43)	86.96 (70.73)	83.60 (67.36)	
CD (P=0.05) Formulation (T) : (2.30) Storage Time (t): (2.30) Formulation x Storage Time (Tx t): (5.63)							

Figures in parentheses are arc sin transformed values

Table 2: Per cent survival of infective juveniles of *Oscheius rugaoensis* in different formulations stored at 30 ^{0}C (Mean of five replications)

Formulations	Survival (%)						Mean
	1st week	2nd week	3rd week	4th week	5th week	6th week	
Talc	99.20 (85.99)	97.80 (82.49)	93.00 (76.39)	86.20 (68.34)	68.20 (59.93)	68.00 (55.69)	85.40 (71.47)
Sawdust	100 (89.78)	100 (89.78)	100 (89.78)	94.00 (77.41)	92.00 (73.77)	87.00 (68.91)	95.50 (81.57)
Alginate gel	100 (89.78)	100 (89.78)	100 (89.78)	94.00 (78.85)	88.00 (70.17)	80.60 (63.90)	93.76 (79.23)
Water dispersible granule (WDG)	90.40 (72.05)	78.40 (62.45)	67.60 (55.41)	63.60 (52.94)	57.80 (49.54)	57.20 (49.17)	69.16 (56.93)
Compost : Charcoal powder mixture (1:1)	100 (89.78)	100 (89.78)	97.20 (82.80)	87.60 (73.77)	73.80 (59.95)	67.20 (55.16)	88.10 (74.57)
Control (water)	100 (89.78)	100 (89.78)	94.80 (79.65)	92.20 (75.63)	87.20 (71.28)	81.20 (64.79)	92.56 (78.49)
Mean	98.26 (86.19)	96.03 (83.40)	92.10 (77.79)	86.26 (71.16)	77.83 (64.11)	73.53 (59.61)	
CD (P=0.05) Formulation (T) : (2.65) Storage Time (t): (2.65) Formulation x Storage Time (T x t): (6.50)							

Figures in parentheses are arc sin transformed values

Per cent survival of *O. rugaoensis* infective juveniles (IJs) was 98.30% in sawdust, and 97.30% in alginate gel up to 6th week of storage at 5^{0}C, whereas per cent survival was less (95.50% and 93.76% respectively) at 30^{0}C up to 6th week of storage. The studies says that energy reserves usually exhausted much faster as storage time increase [2, 3]. Temperature is the most important factor affecting nematode survival in formulations. Alginate based *Steinernema carpocapsae* products were the first to possess room temperature shelf-life and led to increased acceptability of the nematode. *S. carpocapsae* formulated in alginate gel could be stored up to 6-12 months at refrigerated conditions and 1-3 months at room temperature [4]. Similar investigations were made by (Divya *et al*., 2011) [5] on the development of five different formulations of *H. indica*, sawdust, hydrogel, coirdust, talc and sponge and were evaluated its survival at 27 ± 2^{0}C. Sawdust (95%) and hydrogel (85%) formulations were enhanced highest survival followed by coir dust (80%), talc (75%) and sponge (65%) till 5 week period. A maximum shelf-life of more than 11 week periods achieved in hydrogel formulation with 65% of survival than sawdust formulation.

3.2 Soil bioassay

Irrespective of exposure time, the formulations such as talc, sawdust, alginate gel and compost charcoal mixture showed higher mortality of *Galleria* larvae as compared to control (water). Sawdust formulation was found to be most effective on larval mortality of *Galleria* (87.33%) than Alginate gel (82.66%) (Table. 3) at 5^{0}C whereas per cent larval mortality was less (82.66% and 79.33%, respectively) at 30^{0}C (Table.4). Larval mortality of *G. mellonella* by *O. rugaoensis was* 47.66 %, 67.33 %, and 81.66 % at 24 h, 48 h and 72 h respectively at 5^{0}C whereas larval mortality was 43.66%, 64.66 % and 78.00 % at 24 h, 48 h and 72 h respectively at 30 ^{0}C.

Table 3: Per cent larval mortality of *Galleria mellonella* by *Oscheius rugaoensis* in different formulations stored at 5^{0}C in soil bioassay (Mean of five replications)

Formulations	Larval mortality (%)						Mean
	24 hrs	% increase/ decrease over control	48 hrs	% increase/ decrease over control	72 hrs	% increase/ decrease over control	
Talc	40.00 (39.18)	0.00	60.00 (50.99)	20.00 (+ve)	86.00 (72.61)	48.27 (+ve)	62.00 (54.26)
Sawdust	66.00 (54.55)	65.00 (+ve)	96.00 (82.44)	92.00 (+ve)	100 (89.69)	72.41 (+ve)	87.33 (75.56)
Alginate gel	60.00 (50.81)	50.00 (+ve)	88.00 (71.94)	76.00 (+ve)	100 (89.69)	72.41 (+ve)	82.66 (70.81)
Water dispersible granule (WDG)	30.00 (33.08)	25.00 (-ve)	42.00 (40.38)	16.00 (-ve)	58.00 (49.66)	0.00 (+ve)	43.33 (41.04)
Compost : Charcoal powder mixture (1:1)	50.00 (45.00)	25.00 (+ve)	68.00 (55.71)	36.00 (+ve)	88.00 (74.23)	51.72 (+ve)	68.66 (58.31)
Control (water)	40.00 (41.53)		50.00 (45.00)		58.00 (49.66)		49.33 (45.40)
Mean	47.66 (44.03)		67.33 (57.74)		81.66 (70.92)		
CD (P=0.05) Formulation (T): (5.44) Exposure period (t): (3.85) Formulation x Exposure period (T x t): (9.43)							

Figures in parentheses are arc sin transformed values

Table 4: Per cent larval mortality of *Galleria mellonella* by *Oscheius rugaoensis* in different formulations stored at 30^{0}C in soil bioassay (Mean of five replications)

Formulations	Larval mortality (%)						Mean
	24 hrs	% increase/ decrease over control	48 hrs	% increase/ decrease over control	72 hrs	% increase/ decrease over control	
Talc	36.00 (36.82)	0.00	56.00 (48.51)	12.00 (+ve)	80.00 (63.73)	48.18 (+ve)	57.33 (49.68)
Sawdust	62.00 (51.97)	72.22 (+ve)	90.00 (73.56)	80.00 (+ve)	96.00 (82.44)	77.77 (+ve)	82.66 (69.72)
Alginate gel	54.00 (47.35)	50.00 (+ve)	88.00 (71.94)	76.00 (+ve)	96.00 (82.44)	77.77 (+ve)	79.33 (67.24)
Water dispersible granule (WDG)	28.00 (31.88)	22.22 (-ve)	40.00 (38.18)	20.00 (-ve)	56.00 (48.51)	3.70 (+ve)	41.33 (39.85)
Compost : Charcoal powder mixture (1:1)	46.00 (42.69)	27.77 (+ve)	64.00 (53.22)	28.00 (+ve)	86.00 (70.61)	59.25 (+ve)	65.33 (55.51)
Control (water)	36.00 (36.82)		50.00 (45.00)		54.00 (47.35)		46.66 (43.06)
Mean	43.66 (41.25)		64.66 (55.23)		78.00 (65.85)		
CD (P=0.05) Formulation (T) : (4.78) Exposure time (t): (3.38) Formulation x Exposure period (T x t): (8.28)							

Figures in parentheses are arc sin transformed values

Per cent infectivity to *G. mellonella* larva by *O. rugaoensis* was more in sawdust formulation in soil bioassay. Survival and infectivity is conserved at lower temperatures due to the tendency of IJs to be less active [6]. *H. indica* infectivity under formulation was lead to cause 85% and 70% pathogenicity on *Helicoverpa armigera* larvae in hydrogel and sawdust

respectively, exposed for 48 h treated 100 infective juvenile /larva [5].

4. Conclusion

Among various formulations evaluated in the laboratory, sawdust and alginate gel based formulations were the best as maximum numbers of infective juveniles of *O. rugaoensis* were survived without ceasing their pathogenicity on wax moth larvae and these formulations may be useful for the biocontrol of insect pests in the soil. Per cent survival of *Oscheius rugaoensis* infective juveniles (IJs) was 98.30% in sawdust and 97.30% in alginate gel up to 6th week of storage at 5^{0}C, whereas per cent survival was less (95.50% and 93.76 % respectively) at 30^{0}C up to 6th week of storage. Larval mortality of *G. mellonella* by *Oscheius rugaoensis was* 47.66%, 67.33%, and 81.66% at 24 h, 48 h and 72 h respectively at 5^{0}C whereas larval mortality was 43.66%, 64.66% and 78.00% at 24 h, 48 h and 72 h respectively at 30^{0}C.

The present study confirms the scope for formulating *O. rugaoensis* in alginate gel and sawdust. From the economic point of view, sawdust formulation is the best for *O. rugaoensis.* Improved understanding of the nematode behaviour and physiology could lead to the development of better formulations.

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