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EFFECT OF CHEMICAL ADJUVANTS ON SURVIVAL OF ENTOMOPATHOGENIC NEMATODES (*HETERORHABDITIS INDICA*) POINAR AND THEIR INFECTIVITY AGAINST *HELICOVERPA ARMIGERA* (HUB)

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ABSTRACT

Present study on "Effect of chemical adjuvants on survival of Entomopathogenic nematodes (*Heterorhabditis indica*) and their infectivity against *Helicoverpa armigera*". The maximum of 100.00 per cent survival of *H. indica* was observed in the treatment with *H. indica* + APSA 80 (0.1 and 0.5 %), *H. indica* + glycerol (0.1 %), *H. indica* + ujala (0.1 and 0.5 %) and *H. indica* alone and Infectivity of *H. indica* against 3rd instar larvae of *H. armigera* were ranged from 41.11 to 72.22 per cent and the highest infectivity of 72.22 per cent was recorded in the treatments with *H. indica* + ujala (0.1 %) and *H. indica* alone (71.13 %). The four adjuvants and their combinations tested for survival and infectivity of IJs at different concentration, APSA 80 + Glycerol + Ujala @ 0.1 per cent recorded maximum survival (97.78%) as well as infectivity (70.00 %) of IJs. The formulation *H. indica* + APSA 80 + Glycerol + Ujala @ 0.1 per cent adjuvants showed appreciable UV rays protectability (83.33 %) and were considered for tank mixed application.

INTRODUCTION

The use of entomopathogenic nematodes (EPNs) is being explored as a component of integrated pest management (IPM). The impressive attributes of EPN have stimulated strong commercial interest in nematodes as biological insecticides. These attributes include their wide spectrum insecticidal activity, ability to kill their hosts within short period, efficient mass culturing techniques and exemption by EPA for registration. Hence, these insecticidal nematodes are perceived as viable alternatives to chemical insecticides. EPN possesses the qualities of parasitoids/predators and also pathogens like quick kill, broad host range, high virulence, presence of chemoreceptor, amenability for *in-vitro* production, safety to vertebrates, plants and non targets, easy application using standard application equipments, compatibility with many chemical pesticides and genetic diversity (Hussaini 2003).

Heterorhabditids and Steinernematids have mutualistic association with the bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively (Glazer, 1992). The third stage infective juvenile is a free living, non-feeding active stage, which is capable of withstanding adverse climatic conditions and non-availability of the host for a long period. Other advantages include a durable infective stage capable of long term storage, wide distribution and persistence with no evidence of insect immunity, safety to plants and vertebrates, ease of mass production and field application with existing spray application techniques (Poinar, 1979; Kaya and Readon, 1982; Georgis 1992; Glazer and Golberg, 1993).

An effective formulation of EPN provides extended shelf life, ease of handling (Georgis *et al.*, 1994). Entomopathogenic nematodes have been formulated for commercial application in various carriers including clay, activated charcoal, sponge, vermiculite, peat, saw dust, alginate gels and water-dispersible granules along with different adjuvants (Georgis, 1990; Georgis *et al.*, 1994). Shelf life in most formulations is enhanced by the reduction of nematode metabolism through partial desiccation and/or storage at low temperatures e.g 4-15°C. Hence, considering the eco-friendly and biological management of the pests without disturbing the ecosystem, there is a need to develop viable and potential formulations of *H. indica*, Hence, the present investigation on effect of chemical adjuvants on survival of Entomopathogenic nematodes *Heterorhabditis indica* (Poinar) and their infectivity against *Helicoverpa armigera*. (Hub).

MATERIALS AND METHODS

The nucleus culture of Entomopathogenic nematodes, *H. indica* was made available from Dr. Sharad Mohon, Principal Scientist, Division of Nematology, IARI, New Delhi. All glass wares, chemicals and other accessories used during the investigation were of high quality. Entomopathogenic nematodes, *H. indica* were reared on last instar larvae of wax moth, *Galleria mellonella* at 25 °C as per the procedure given by Wooding and Kaya (1988). Nematodes were stored in distilled water at 15 °C and allowed to acclimatize at ambient room temperature (23-25 °C) for 24 h before use.

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Effect of chemical adjuvants on per cent survival of EPN (*H. indica*) and infectivity against *Helicoverpa armigera*

Four different chemical adjuvants viz., APSA-80, Glycerol, Actiwet and Ujala (0.1, 0.5, 1.0, 1.5 and 2 % each) were mixed individually in optimum concentration of liquid culture *H. indica* (200 IJs/ml) and 20 formulations were prepared. These formulations of *H. indica* were tested for their effect on survival and infectivity. The experiment was carried out in C.R.D. with three replications. Survival of IJs of *H. indica* was recorded at 12, 24 and 48 h at 27 + 2°C. The mean data on per cent survival of IJs was worked out. Straight and immovable nematodes were considered as dead whereas, moving individuals irrespective of activity were considering as live. The infectivity was also tested against 3rd instar larvae of *H. armigera* by applying different concentrations of adjuvants in distilled water with 200 IJs/ml. The infectivity was observed at 12, 24 and 48 h and data were subjected to statistical analysis and the promising formulations were considered for further study.

Effect of combination of chemical adjuvants on per cent survival of EPN (*H. indica*)

Different chemical adjuvants at various concentrations were combined with *H. indica* and evaluated for their effect on survival and infectivity. The chemical adjuvants at different concentrations viz., APSA 80, Glycerol and Ujala (0.1, 0.5, 1.0 and 1.5 %, each), and Actiwet (0.1 and 0.5 %) were selected and combined with each other in optimum concentration of aqua suspension (200 IJs per ml) of *H. indica* and six liquid formulations with multiple adjuvants were prepared. These six formulations were tested for their effect on survival and infectivity of *H. indica* in C.R.D. with three replications.

Effect of UV rays on per cent survival of the *H. indica* in

different advanced stage formulations

The promising test formulations emerged out comprising of *H. indica* + APSA 80 + Gly + Ujala (0.1, 0.5, 1.0 and 1.5 %) and *H. indica* + Actiwet + Gly + Ujala (0.1 and 0.5 %) with UV protecting Ujala and control without adjuvants i.e. *H. indica* alone (aqua suspension) were evaluated in C.R.D. with 3 replications for their protecting ability under UV rays. (Ahmad, R. et al., 2009) The respective quantities as per concentrations of adjuvants were added to optimum concentration of *H. indica* aqua suspension culture to prepare different formulations. Each formulation was kept in 50 ml beaker and was exposed to UV rays for 1, 3, 7 and 10 h. The distance between exposed suspensions and UV light source was 0.3 m. one ml of such exposed formulation was added in 9 ml distilled water and observed dead and live nematodes up to 10 hours. Then data were subjected to statistical analysis. Promising formulations were considered as adjuvants for tank mix application.

RESULTS AND DISCUSSION

Effect of chemical adjuvants on per cent survival of EPN (*H. indica*)

The per cent survival of EPN was in the range of 97.67 to 100.00, 76.67 to 100.00 and 76.67 to 100.00 per cent at 12, 24 and 48 HAI, respectively. Hence it is found that the trend of mortality was more or less similar in all the treatments.

The effects of chemical adjuvants on per cent survival of EPN (*H. indica*) recorded at 12, 24, and 48 hours after incubation in Table 1, it is noted that the mean cumulative per cent survival of *H. indica* showed that maximum of 100.00 per cent survival of *H. indica* was observed in the treatment with *H. indica* + APSA 80 (0.1 and 0.5 %), *H. indica* + glycerol (0.1 %), *H. indica* + ujala (0.1 and 0.5 %) and *H. indica* alone followed

Table 1: Effect of chemical adjuvants on per cent survival of EPN (*H. Indica*)

Tr. No.	Treatment	Survival of <i>H. indica</i> (%)			
		12 HAI**	24 HAI	48 HAI	Mean
T ₁	<i>H. indica</i> + APSA 80 @ 0.1 %	100.00(90.00)*	100.00(90.00)	100.00(90.00)	100.00(90.00)
T ₂	<i>H. indica</i> + APSA 80 @ 0.5 %	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
T ₃	<i>H. indica</i> + APSA 80 @ 1.0 %	100.00(90.00)	99.00(86.67)	95.67(80.53)	98.22(85.73)
T ₄	<i>H. indica</i> + APSA 80 @ 1.5 %	100.00(90.00)	98.00(82.05)	91.67(74.10)	96.55 (82.05)
T ₅	<i>H. indica</i> + APSA 80 @ 2.0 %	98.00(83.78)	90.67(72.65)	90.67(72.67)	93.11(76.36)
T ₆	<i>H. indica</i> + Acti wet @ 0.1 %	100.00(90.00)	98.00(83.44)	98.00(83.45)	98.67 (85.63)
T ₇	<i>H. indica</i> + Acti wet @ 0.5 %	100.00(90.00)	96.22(81.22)	94.33(76.83)	96.85 (82.68)
T ₈	<i>H. indica</i> + Acti wet @ 1.0 %	99.67 (88.08)	85.67(68.07)	85.65(68.07)	90.33 (74.74)
T ₉	<i>H. indica</i> + Acti wet @ 1.5 %	98.67(84.76)	84.00(68.23)	84.00(68.23)	88.89 (73.74)
T ₁₀	<i>H. indica</i> + Acti wet @ 2.0 %	97.67(82.98)	76.67(61.39)	76.67(61.39)	83.67(68.58)
T ₁₁	<i>H. indica</i> + Glycerol @ 0.1 %	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
T ₁₂	<i>H. indica</i> + Glycerol @ 0.5 %	100.00(90.00)	98.67(85.63)	91.67(74.10)	96.78(83.24)
T ₁₃	<i>H. indica</i> + Glycerol @ 1.0 %	100.00(90.00)	97.67(83.24)	90.67(72.50)	96.11(81.91)
T ₁₄	<i>H. indica</i> + Glycerol @ 1.5 %	100.00(90.00)	96.78(82.98)	90.67(72.50)	95.81 (81.82)
T ₁₅	<i>H. indica</i> + Glycerol @ 2.0 %	97.67(82.98)	77.67(62.38)	77.67(62.38)	85.11(71.58)
T ₁₆	<i>H. indica</i> + Ujala @ 0.1	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
T ₁₇	<i>H. indica</i> + Ujala @ 0.5	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
T ₁₈	<i>H. indica</i> + Ujala @ 1.0	100.00(90.00)	100.00(90.00)	97.43(80.48)	99.14(84.68)
T ₁₉	<i>H. indica</i> + Ujala @ 1.5	100.00(90.00)	99.00(84.26)	95.50(77.75)	96.55(82.05)
T ₂₀	<i>H. indica</i> + Ujala @ 2.0	99.00(84.26)	94.67(72.65)	92.00(73.57)	95.50(76.82)
T ₂₁	<i>H. indica</i> alone	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
	SE ±	1.58	3.41	3.53	2.84
	CD at 5%	4.56	9.82	10.17	8.18

* Figures in the parenthesis are arc sin values ** HAI - Hours after inoculation

Table 2: Effect of chemical adjuvants on per cent infectivity of EPN (*H. Indica*) against 3rd instar larvae of *H. armigera*

Tr. No.	Treatment	Infectivity of <i>H. indica</i> (%)			Mean
		24 HAI**	48 HAI	72 HAI	
T ₁	<i>H. indica</i> + APSA 80 @ 0.1 %	46.67(43.08)*	66.67(54.78)	100.00(90.00)	71.11(62.62)
T ₂	<i>H. indica</i> + APSA 80 @ 0.5 %	43.33(41.15)	63.33(52.78)	100.00(90.00)	68.89(61.31)
T ₃	<i>H. indica</i> + APSA 80 @ 1.0 %	40.00(39.23)	60.00(50.77)	93.33(77.71)	64.44(55.9)
T ₄	<i>H. indica</i> + APSA 80 @ 1.5 %	36.67(37.22)	56.67(48.85)	90.00(75.00)	61.11(53.69)
T ₅	<i>H. indica</i> + APSA 80 @ 2.0 %	33.33(35.01)	43.33(41.15)	76.67(61.22)	51.11(45.79)
T ₆	<i>H. indica</i> + Acti wet @ 0.1 %	40.00(39.15)	60.00(50.85)	93.33(77.71)	64.44(55.9)
T ₇	<i>H. indica</i> + Acti wet @ 0.5 %	36.67(37.22)	56.67(48.85)	90.00(71.57)	61.11(52.55)
T ₈	<i>H. indica</i> + Acti wet @ 1.0 %	26.67(31.00)	43.33(41.15)	76.67(61.22)	48.89(44.46)
T ₉	<i>H. indica</i> + Acti wet @ 1.5 %	23.33(28.78)	36.67(37.22)	76.67(61.71)	45.56(42.57)
T ₁₀	<i>H. indica</i> + Acti wet @ 2.0 %	20.00(26.57)	30.00(32.22)	73.33(59.00)	41.11(39.59)
T ₁₁	<i>H. indica</i> + Glycerol @ 0.1 %	46.67(43.08)	63.33(53.07)	100.00(90.00)	67.78(60.67)
T ₁₂	<i>H. indica</i> + Glycerol @ 0.5 %	43.33(41.15)	60.00(50.85)	96.67(83.86)	64.44(57.31)
T ₁₃	<i>H. indica</i> + Glycerol @ 1.0 %	40.00(38.86)	56.67(48.93)	93.33(77.71)	61.11(53.95)
T ₁₄	<i>H. indica</i> + Glycerol @ 1.5 %	36.67(37.22)	53.33(47.01)	90.00(71.57)	57.78(52.46)
T ₁₅	<i>H. indica</i> + Glycerol @ 2.0 %	26.67(31.00)	33.33(35.22)	66.67(55.86)	42.22(40.69)

Table 3: Effect of combination of different chemical adjuvants on per cent survival of *H. indica*

Tr. No.	Treatment	Survival of <i>H. indica</i> (%)			Mean
		12 HAI**	24 HAI	48 HAI	
T ₁	<i>H. indica</i> + APSA 80 + Gly*** + Ujala @ 0.1 %	99.33(87.33)*	97.33(81.07)	96.67(79.66)	97.78(82.67)
T ₂	<i>H. indica</i> + APSA 80 + Gly + Ujala @ 0.5 %	98.67(84.60)	95.00(79.92)	94.33(79.11)	96.00(81.20)
T ₃	<i>H. indica</i> + APSA 80 + Gly + Ujala @ 1.0 %	95.67(78.30)	92.33(74.97)	88.00(70.38)	92.00(74.54)
T ₄	<i>H. indica</i> + APSA 80 + Gly + Ujala @ 1.5 %	94.33(77.00)	89.33(72.00)	83.67(66.53)	89.11(71.84)
T ₅	<i>H. indica</i> + Actiwet + Gly + Ujala @ 0.1 %	95.67(78.50)	91.00(72.67)	88.00(69.88)	91.56(73.68)
T ₆	<i>H. indica</i> + Actiwet + Gly + Ujala @ 0.5 %	95.00(77.33)	87.67(70.03)	79.33(64.28)	87.33(70.52)
T ₇	<i>H. indica</i> alone	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00(90.00)
SE ±		3.77	4.03	2.32	3.40
CD at 5%		11.44	12.22	7.05	10.23

* Figures in the parenthesis are arc sin values; ** HAI- hours after inoculation; *** Gly – Glycerol

Table 4: Effect of combination of different chemical adjuvants on per cent infectivity of *H. indica* against III instar larvae of *H. armigera*

Tr. No.	Treatment	Infectivity of <i>H. indica</i> (%)			Mean
		24 HAI**	48 HAI	72 HAI	
T ₁	<i>H. indica</i> + APSA 80 + Gly*** + ujala @ 0.1	40.00(39.15)*	70.00(57.00)	100.00(90.00)	70.00(62.05)
T ₂	<i>H. indica</i> + APSA 80 + Gly + ujala @ 0.5	36.67(37.22)	66.67(55.07)	96.67(83.86)	66.67(58.72)
T ₃	<i>H. indica</i> + APSA 80 + Gly + ujala @ 1.0	30.00(33.21)	46.67(43.08)	90.00(71.57)	55.56(49.28)
T ₄	<i>H. indica</i> + APSA 80 + Gly + ujala @ 1.5	26.67(30.79)	43.33(41.15)	80.00(63.44)	50.00(45.13)
T ₅	<i>H. indica</i> + Actiwet + Gly + ujala @ 0.1	33.33(35.22)	63.33(52.78)	93.33(77.71)	63.33(55.23)
T ₆	<i>H. indica</i> + Actiwet + Gly + ujala @ 0.5	26.67(31.00)	60.00(50.85)	86.67(68.86)	57.78(50.23)
T ₇	<i>H. indica</i> alone	46.67(43.08)	76.67(61.22)	100.00(90.00)	74.44(64.77)
SE ±		2.56	3.17	3.44	3.05
CD at 5%		7.78	9.61	10.43	9.27

* Figures in the parenthesis indicate arc sin transformed values; ** HAI - hours after inoculation, *** Gly – Glycerol

by treatments with *H. indica* + ujala @ 1.0 per cent (99.14 %), *H. indica* + actiwet @ 0.1 per cent (98.67 %), *H. indica* + APSA 80 @ 1.0 per cent (98.22 %), *H. indica* + actiwet @ 0.5 per cent (96.85 %), *H. indica* + glycerol @ 0.5 per cent (96.78 %), *H. indica* + APSA 80 @ 1.5 per cent (96.55 %), *H. indica* + ujala @ 1.5 per cent (96.55 %) and *H. indica* + glycerol @ 1.5 per cent (95.81 %). Lowest of 85.11 per cent survival of *H. indica* was noticed in the treatment with glycerol (2.0 %).

Effect of chemical adjuvants on per cent infectivity of EPN (*H. indica*) against 3rd instar larvae of *H. armigera*

The result pertaining to the effect of chemical adjuvants on per cent infectivity of EPN (*H. indica*) at 24, 48 and 72 hours

after incubation (HAI) in Table 2 revealed that the mean cumulative infectivity of *H. indica* against 3rd instar larvae of *H. armigera* was ranged from 41.11 to 72.22 per cent and the highest infectivity of 72.22 per cent were recorded in the treatments with *H. indica* + ujala (0.1 %) and *H. indica* alone followed by *H. indica* + APSA 80 (0.1 %) with 71.11 per cent infectivity and *H. indica* + APSA 80 and *H. indica* + ujala (0.5 %, each) with 68.89 per cent infectivity each, respectively, *H. indica* + glycerol (0.1 %) with 67.78 per cent infectivity, *H. indica* + ujala (1.0 %) with 65.56 per cent infectivity, *H. indica* + APSA 80 (1.0 %) with 64.44 per cent infectivity, *H. indica* + actiwet (0.1 %) with 64.44 per cent infectivity, *H. indica* + glycerol (0.5 %) with 64.44 per cent infectivity, *H. indica* +

Table 5: Effect of UV rays on per cent survival of *H. indica* in different advanced stage formulations

Tr.No	Treatment	Per cent IJs survival after UV rays exposure at different hours				Mean
		1 h**	3 h	7 h	10 h	
T1	<i>H. indica</i> + APSA 80 + Gly*** + Ujala @ 0.1	85.67(67.82)*	84.33(66.79)	82.33(65.20)	83.00(65.67)	83.83(66.37)
T2	<i>H. indica</i> + APSA 80 + Gly + ujala @ 0.5	86.00(68.06)	85.00(67.26)	83.67(66.22)	83.67(66.20)	84.58(66.93)
T3	<i>H. indica</i> + APSA 80 + Gly + ujala @ 1.0	88.33(70.40)	85.33(67.59)	85.33(67.51)	84.33(66.77)	85.83(68.06)
T4	<i>H. indica</i> + APSA 80 + Gly + ujala @ 1.5	91.33(72.98)	88.67(70.35)	87.33(69.19)	86.00(68.09)	88.33(70.15)
T5	<i>H. indica</i> + Actiwet + Gly + ujala @ 0.1	82.33(65.16)	75.33(60.23)	69.67(56.62)	62.00(51.98)	72.33(58.49)
T6	<i>H. indica</i> + Actiwet + Gly + ujala @ 0.5	84.67(66.99)	74.67(59.86)	70.67(57.24)	66.00(54.36)	74.00(59.61)
T7	<i>H. indica</i> alone	80.00(63.47)	73.00(58.71)	64.00(53.24)	58.00(49.63)	68.75(56.26)
SE ±		1.7	1.5	1.9	1.7	1.7
CD at 5%		5.13	4.61	5.77	5.19	5.17

* Figures in the parenthesis are arc. sin transformed values; ** h - Hours *** Gly - Glycerol

APSA 80 and *H. indica* + ujala (1.5 %, each) with 61.11 per cent infectivity each *H. indica* + actiwet (0.5 %) with 61.11 per cent infectivity and *H. indica* + glycerol (1.5 %) with 57.78 per cent infectivity. Minimum per cent infectivity (41.11 %) was noticed in the treatment with acti wet (2.0%).

Effect of combination of chemical adjuvants on per cent survival of EPN (*H. indica*)

The effect of combination of different chemical adjuvants on per cent survival of *H. indica* showed that maximum of cent per cent survival of *H. indica* was recorded in the treatment with *H. indica* alone which was followed by treatment with *H. indica* + APSA 80 + Gly + ujala (0.1 and 0.5 %) with 97.78 and 96.00 per cent survival, respectively in table 3.

The results were in the argument with Somvanshi *et al.* (1998) reported that higher levels of the adjuvant APSA 80, *i.e.* 0.3, 1.0 gave significant but low mortality of entomopathogenic nematodes IJs 3.00, 5.80 and 9.10 per cent mortality after incubation. Earlier studies on some other adjuvants Triton - X 100, Glycerol, Croduvant, Crovol 127 and L 40 by Mason *et al.* (1998), reported that the increased incubation time of IJs in the adjuvant solution generally resulted in a slight reduction in survival of IJ. The reason for significant high mortality of IJs at higher levels of concentration and increased time of observation might be attributed to direct toxicity of the adjuvant to IJs.

Effect of combination of chemical adjuvants on percent infectivity of EPN (*H. indica*) against *H. armigera*

Effect combination of chemical adjuvants on per cent infectivity of *H. indica* against 3rd instar larvae of *H. armigera* was ranged from 50.00 to 74.44 per cent and the highest infectivity of 74.44 per cent was recorded in the treatment with *H. indica* alone followed by the treatment with *H. indica* + APSA 80 + Gly + ujala (0.1 %) with 70.00 per cent infectivity and *H. indica* + APSA 80 + Gly + ujala (0.5 %) with 66.67 per cent infectivity in table 4.

Thus the results obtained are on line of Somvanshi *et al.* (1998) and Mason *et al.* (1998) that the infectivity of the IJs to the larvae of diamond back moth and wax moth in terms of mortality and intensity of infection was affected by the addition of the adjuvants compared with IJs in water alone. This is because of sharp reduction in the infectivity of *H. indica* might be attributed to the significant mortalities in IJs due to direct toxicity of adjuvant at concentration higher than the recommended one, for prolonged incubation time.

Effect of UV rays on per cent survival of the *H. indica* in different advanced stage formulations

Per cent survival of *H. indica* with combination of different adjuvants in culture medium after exposing to UV light for 1, 3, 7 and 10 h showed that the highest per cent survival (88.33 %) was found in treatment with *H. indica* + APSA 80 + Gly + ujala at 1.5 per cent and it was at par with *H. indica* + APSA 80 + Gly + ujala @ (1.0, 0.5 and 0.1 %) which recorded 85.83, 84.58 and 83.83 per cent survival of IJs, respectively in table 5.

The results are in line with those reported by Ahmad *et al.* (2009) that the formulation was improved by the addition of glycerin as anti desiccant and Ujala as UV protectant under field conditions.

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