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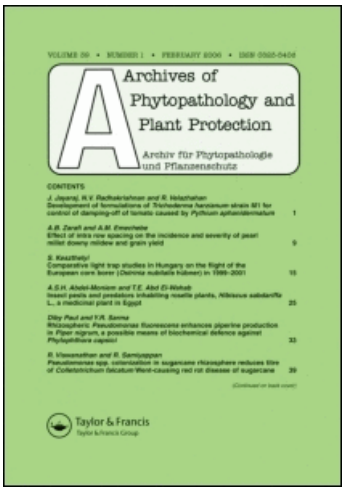

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R. Ahmad [a]; M. Abid Hussain [a]; S. S. Ali [a]; R. Pervez [a]
[a] Division of Crop Protection, Indian Institute of Pulses Research, Kanpur, India

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Survival of *Steinernema masoodi* and *S. carpocapsae* (Rhabditida: Steinernematidae) on pigeonpea and chickpea after foliar application

R. AHMAD, M. ABID HUSSAIN, S. S. ALI, & R. PERVEZ

Division of Crop Protection, Indian Institute of Pulses Research, Kanpur – 208024, India

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Abstract
The survival of *Steinernema masoodi* and *S. carpocapsae* (Rhabditida: Steinernematidae) was investigated after foliar application on pigeonpea and chickpea twigs, respectively, at flowering and fruiting stage. The concentration used was 2500 infective juveniles (IJs)/ml water for both the species. On pigeonpea, the mean number of IJs of *S. masoodi* found alive were 303.4, 158.4 and 51.6 after 0, 30 and 60 minutes of spray in evening hours whereas 236.1, 44.4 and 6.8 IJs were found alive when sprayed in morning hours, respectively. *S. masoodi* survival at 30 minutes post-spray in the morning was on par with 60 minutes post-spray in the evening hours. On chickpea, the mean numbers of IJs of *S. carpocapsae* were 165.4, 65.8, 4 and 0 at 0, 1, 2 and 3 h post-spray in the morning hours whereas in the evening spray, 159.4, 111.8, 83.8 and 11.4 IJs found alive at 0, 1, 2 and 3 h post-spray, respectively. Overall, nematode survival in the evening hours was higher compared to morning spray at a given time. Addition of glycerine and UV retardant improved the survival of nematode. Results indicated that survival rate of IJs decreased fast and viability remained up to 3 h and in evening hours very few nematodes remained alive. Serious attempts are needed to improve the survival of nematodes after foliar spray by adding efficient adjuvant, humectant, antidesiccant and/or UV retardant for the management of aerial insect pests.

Keywords: *Survival, entomopathogenic nematodes,* Steinernema masoodi, Steinernema carpocapsae, *foliar application,* Cajanus cajan, Cicer arietinum

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) and chickpea (*Cicer arietinum* L.) are important grain legumes in India and provide nutritious food, feed and fodder and constitute an integral component of subsistence farming system of the country. Among the various biotic constraints, the infestation and damage caused by insect pests is one of the major constraints towards their low production. About 20 insect pests have been reported to be of major importance at various growth stages of pigeonpea and chickpea inflicting heavy yield losses (Kumar & Nath 2003; Kooner et al. 2005). Average yield loss due to insect pests in chickpea has been estimated as 29.2% whereas in pigeonpea often exceeds 50% (Kooner et al. 2005).

Correspondence: R. Ahmad, Division of Crop Protection, Indian Institute of Pulses Research, Kanpur – 208024, India.
Fax: +91 512 257 2582. E-mail: rahmad@iipr.ernet.in

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Entomopathogenic nematodes (EPN) of the family Steinernematidae are recognized as potent bioagents against some of the agriculturally important insect pests (Kaya & Gaugler 1993; Ali et al. 2005a, 2005b). A number of attributes of EPN, such as host-finding ability, rapid death of host insect after infection (24–48 h), nontoxicity to vertebrates and environmental safety, have generated interest in their use as biopesticides. Soil application of EPN through irrigation is a successful method against soil insect pests (Feaster & Steinkraus 1996), however, use of EPN to manage insect pests feeding on aerial parts poses a considerable challenge as aboveground conditions are detrimental to nematodes (Arthurs et al. 2004). Infective juveniles (IJs) get inactivated quickly and are sensitive to extremes of physical environment, particularly rapid desiccation (Womersley 1990), high temperature (Grewal et al. 1994), lethal UV radiation (Gaugler et al. 1992), and difficulty in establishing attraction gradients (Glazer 1992). Particularly, foliar application of EPN against aerial insect pests at 35–40°C needs to be resolved by improving their survival and efficacy. Efforts have been made to increase the survival of EPN through addition of adjuvant to minimize the above mentioned detrimental factors. An attempt was made to study the survival of *Steinernema masoodi* on pigeonpea and *S. carpocapsae* on chickpea after foliar spray at fruiting stage through introducing adjuvant, namely, UV retardant (fabric whitener) and glycerine at high temperature regimes.

Materials and methods

Nematodes and insect culture

Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), required for in vivo production of EPN, was reared on a semi-synthetic diet as per procedure described by Ali et al. (2005b). *S. masoodi* and *S. carpocapsae* were multiplied on the last instar larvae of *G. mellonella* and freshly harvested IJs was used in the present study.

EPN survival on pigeonpea

Foliar application of *S. masoodi* was done on an early maturing variety of pigeonpea, UPAS 120, when the crop was at fruiting stage. About 8 ml of nematode suspension (concentration: 2500 IJs/ml) was sprayed on the apical portion of each plant with a hand-compressed sprayer in an area of 20 m^2 at morning (7:00 am) and evening (4:30 pm) hours. Ten leaves and two pods per plant were plucked after 0, 30 and 60 minutes of spray and dipped in 100 ml distilled water and left for 1 hour before counting the number of live IJs under a binocular microscope. There were 18 treatments, viz., three formulations (EPN alone, EPN + Glycerine 1%, EPN + Glycerine 1% + Ujala 0.01% as UV retardant) × three observation periods (0, 30 and 60 minutes) × 2 spray schedule (morning and evening hours) and replicated seven times. Temperature and prevailing RH were also recorded simultaneously.

EPN survival on chickpea

Foliar application of *S. carpocapsae* was applied on the chickpea variety, SAKI 95–16, when the crop was at fruiting stage. Liquid EPN suspension containing 2500 IJs/ml + glycerine 1% + Ujala 0.01% as UV retardant was prepared in water spray. Sodium bicarbonate (0.5%) was also added to nullify the harmful effect of malic acid on chickpea foliage. Spraying was done in an area of 3 m × 3 m plot in the morning (6:30 am) and evening (5:30 pm) hours. Two twigs along with pods per plant were cut with scissors after 0, 1, 2 and 3 h post-spray and

dipped in 100 ml distilled water and left for 1 hour in the morning spray and overnight in case of evening spray before the final counts were made. There were eight treatments: four observation periods (0, 1, 2 and 3 h) × 2 spray timings (morning and evening) and these were replicated five times. Records of temperature and prevailing RH were also taken simultaneously.

Statistical analysis

Data on the survival of the nematode were analyzed using factorial ANOVA and means were separated using LSD. Differences among means in experiments were considered significant at $p < 0.05$.

Results

EPN survival on pigeonpea

In the morning sprays, the *S. masoodi* IJs population reduced drastically from an initial 236.1 to 44.4 within 30 minutes and only a few nematodes could survive after 60 minutes (18.8 and 2.9% survival, respectively, Table I). The population of *S. masoodi* IJs was maximum (mean: 303.4) immediately after the evening spray however a significant reduction (mean: 158.4 and 51.6) was recorded after 30 and 60 minutes and resulted in 52.2 and 17.0% survival from the initial population, respectively. Nematode survival after 30 minutes in morning spray was on par with 60 minutes post-spray in the evening. Thus, spraying during evening hours was found superior with respect to the EPN survival over the morning spray schedule. Addition of adjuvants was used to nullify the effect of external weather factors, like, desiccation and UV radiation to some extent. It was interesting to note that addition of glycerine has always resulted in lower EPN survival at any given exposure period. But when UV retardant was also incorporated, it invariably gave rise to higher EPN survival than the other two treatments irrespective of time elapsed or period of spray. In morning spray, there were 258.6 IJs in this treatment as against 240.4 or 209.4 IJs. Similarly, spray during the evening hours resulted in retaining 329.1 IJs on the leaf surface as against 275.8 or 305.3 IJs in the EPN alone or EPN + glycerine combination.

EPN survival on chickpea

Survival of *S. carpocapsae* IJs when sprayed on chickpea was maximum (mean IJs count: 159.4 and 165.4) in the morning and evening sprays and were on par (Table II). Though with passing of time, the survival of nematode decreased but mortality rate was higher in the morning than evening spray. Three-hour post-spray population of IJs was 11.4 in the evening spray as compared to nil in the morning spray, however, the population was on par with the IJs population 2 h after the morning spray.

Discussion

In a pest management program utilizing EPN as a component, some special considerations are needed. EPN though very effective are delicate and need special care and have to be provided with additives in the form of antidesiccant, UV retardant, humectant, etc. to retain their activity for more duration for effective management. Ultraviolet radiation and dehydration are considered prime mortality factors resulting in 40 – 80% mortality or even

Table I. Survival of *Steinernema masoodi* after spray on pigeonpea.

Spraying schedule (s)	Observation taken after spray (minutes) (t)	Temp. (°C)	Relative humidity (%)	EPN formulation (n)						Grand mean of live IJs (% survival)
				EPN alone		EPN + Glycerine		EPN + Glycerine + Ujala		
				Average no. of live IJs	% survival	Average no. of live IJs	% survival	Average no. of live IJs	% survival	
Morning	0	25.0	75	240.4^{bcd}	–	209.4^{cd}	–	258.6^{abc}	–	236.1 (–)
	30	28.0	71	45.8^{g}	19.1	31.7^{g}	15.1	55.7^{g}	21.5	44.4 (18.8)
	60	30.5	65	16.1^{gh}	6.7	0.3^{h}	0.1	4.0^{h}	1.5	6.8 (2.9)
Evening	0	25.0	58	275.8^{abc}	–	305.3^{ab}	–	329.1^{a}	–	303.4 (–)
	30	21.5	60	148.8^{ef}	53.9	142.8^{ef}	46.8	183.6^{de}	55.8	158.4 (52.2)
	60	17.0	63	37.4^{g}	13.6	68.1^{g}	22.3	49.4^{gh}	15.0	51.6 (17.0)

Different letters among means were considered significant at $p < 0.05$.
C.V. = 33.37%

	CD ($p = 0.05$)	SED
Spraying schedule (s)	15.74	7.93
Time elapsed post-spray (t)	19.27	9.72
Nematode formulation (n)	19.27	9.72
s × t	27.26	13.74
s × t × n	47.22	23.81

Table II. Survival of *Steinernema carpocapsae* after spray on chickpea.

Spraying schedule	Observation taken after spray (hours)	Temp. (°C)	Relative humidity (%)	Average no. of live IJs	% Survival
Morning	0	26.0	71	165.4[a]	–
	1	27.5	69	65.8[c]	39.8
	2	29.0	67	4.0[d]	2.4
	3	32.0	64	0[d]	0
Evening	0	24.0	78	159.4[a]	–
	1	23.5	81	111.8[b]	70.1
	2	22.0	85	83.8[c]	52.6
	3	22.5	87	11.4[d]	7.1
	C.V.			25.5%	
	SED			11.5	
	CD ($p = 0.05$)			23.5	

Different letters among means were considered significant at $p < 0.05$.

more (Smits 1996). In the present study, nematode survival was found lower in the morning spray on pigeonpea foliage on which 2.9% population survived 1 h post-spray than nematode survival ability at evening hours (17.0%). This may be attributed to the increase in solar radiation, temperature from 25.0 to 30.5°C and decrease in relative humidity from 75 to 65%. The reverse was the case with the spray in the evening where higher EPN survival was recorded. Both the factors acted negatively on nematode survival. Among the two adjuvants, UV retardant seemed to have performed better as more survival of EPN was observed irrespective of time elapsed between spray and observation.

On the basis of observation on pigeonpea, another trial was laid down on chickpea, where desiccation and inactivation of *S. carpocapsae* were found to be less, and so much so that 39.8 and 70.1% population survived after 1 h of spray while their survival prolonged for 2 h when sprayed during the evening hours at 22°C. Gaugler et al. (1992) concluded that 60 minutes of exposure to direct sunlight inactivated *S. carpocapsae*. During the morning spray, the temperature gradually increased from 26.0 to 32.0°C within 3 h and humidity decreased from 71 to 64% (Table II). Contrary to this, the corresponding figures for the evening spray were decreasing temperature from 24.0 to 22.5°C and increase in RH from 78 to 87% (Table II). This has resulted in more survival of *S. carpocapsae* on chickpea leaf surface even after 3 h post-spray (0 and 11.4 IJs). Glazer (1991) reported that the survival of IJs of *S. carpocapsae* reduced to 20% after 4 h and to 0% after 8 h at 50–70% RH. Both the experiments on pigeonpea and chickpea suggest that the effects of sunlight, harmful UV rays or high temperature can be minimized by applying the nematodes at dusk as survival of both the species in the evening hours was better than the morning spray schedule. However, maintaining high humidity (>80% RH) and free water on the leaf surfaces is more difficult to achieve especially in dry farming ecosystems such as pigeonpea and chickpea.

Prabhuraj et al. (2005) recorded glycerol 0.1% as the most appropriate antidesiccant resulting in 81.2% survival of *Heterorhabditis indica* after 2 h of foliar spray on chickpea foliage but survival reduced drastically after 4 h under field conditions (12–26°C with 5–60% RH). In the present study, with the passing of time, there was a drastic reduction in the *S. masoodi* population in all the treatments indicating that glycerine 1% and UV retardant 0.01% were not very effective in protecting the nematode survival beyond 3 h post-spray in the morning but effective to some extent in the evening. It suggests that some new molecules acting as

adjuvant, humectant, antidesiccant and/or UV retardant have to be incorporated which, if used along with nematode, can prolong their survival on foliage.

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