

## Superabsorbent hydrogels for efficient biocontrol of root knot nematodes for healthy tomato nursery

Greenhouses are often engaged in growing high-quality vegetables throughout the year, with optimization of inputs such as nutrients and water. Soil pathogens pose a serious threat to greenhouse cultivation of crops and hence it is imperative to manage them. An important way to keep the soil pathogens in check is by incorporating bioagents which have the ability to keep the pathogens at bay in an eco-friendly manner. In greenhouses, moisture stress condition may be a constraint for establishment of bioagents in the soil. Under the greenhouse, seedling mortality, especially shortly after transplanting, occurs due to the inability of seedlings to maintain adequate hydration or through biotic stresses exerted by soil-borne pathogens. One method of supplying additional water is by the use of superabsorbent hydrogels. These are natural or synthetic, organic or inorganic polymers which swell upon absorption of water and enhance both the nutritional and water status of plants. The use of such hydrophilic polymers increases the amount of moisture available in the root zone, thereby permitting longer intervals between irrigations<sup>1</sup>. It also leads to increased water-use efficiency since the water that would have otherwise leached beyond the root zone stays captured. The hydrophilic polymers are effective in increasing the water-holding capacity, decreasing deep percolation, and reducing evaporation losses in sandy soils<sup>2</sup>. In the soil, they swell as gel entities and store water as well as plant nutrients dissolved in the water. They aid in making the soil permeable and aerated, thereby helping the roots to grow vigorously. They also contribute by decreasing water and nutrient losses due to seepage, evaporation and surface run-off. Hydrophilic polymers can build an additional water reservoir for the plant-soil system and thereby reduce water stress in plants<sup>3</sup>. The current experiment was aimed at comparing two hydrogels for their water retention capacity as well as the added advantage they had on better multiplication of biocontrol agents, for managing soil-borne pathogens, especially root knot nematode population, with the overall objective of growing healthier tomato seedlings in the greenhouse.

The experiment was conducted in the polyhouse at the Centre for Protected Cultivation Technology, Indian Agricultural Research Institute (IARI), New Delhi. Sterilized, nursery medium which is a soil-less culture mix comprising coco-peat, perlite and vermiculite in the 3 : 1 : 1 ratio was used in the experiment. Two superabsorbent hydrogels, one developed at IARI, New Delhi (Gel-A) and the other a commercial one (Gel-B) were used for comparison of moisture retention in this study. Each of the two hydrogels was mixed with the potting mixture at the rate of 0.5 mg/cell (100 mg/1 g medium). A nursery plug tray of 180 cells was selected for the experiment, which was further divided into three blocks. In each block with 60 cells, Gel-A + potting mixture, Gel-B + potting mixture, and only potting mixture (control) were filled respectively. Next, 5 ml of water was dispensed into each cell. Five replications were maintained for each treatment. After 24 h, potted-mixture samples of the five cells from each block were collected individually. Each cell sample was put in a petri dish and the initial weight was recorded. The weighed samples were dried for 3 h in a hot-air oven at 120°C. After drying for the stipulated time, the petri dishes were cooled to room temperature and the final weight was recorded. The moisture percentage was calculated using the formula

$$\text{Moisture \%} = (B - C)/(B - A) \times 100,$$

where *A* is the weight of the petri dish, *B* the weight of the petri dish + sample before drying, and *C* the weight of the petri dish + sample after drying.

Percentage of moisture was recorded at an interval of 24, 48, 72, 168, 360 and 720 h. With the same hydrogels (Gel-A and Gel-B) and two bioagents (*Trichoderma harzianum* and *Pseudomonas fluorescens*), another experiment was set up in the greenhouse nursery. The experimental design consisted of three replicates of nine treatments in a randomized block design (RBD). The treatments were as follows:

T1 – Nursery medium + Gel-A + *T. harzianum* + nematode inoculum;

T2 – Nursery medium + Gel-A + *P. fluorescens* + nematode inoculum;

T3 – Nursery medium + Gel-A + nematode inoculum;

T4 – Nursery medium + Gel-B + *T. harzianum* + nematode inoculum;

T5 – Nursery medium + Gel-B + *P. fluorescens* + nematode inoculum;

T6 – Nursery medium + Gel-B + nematode inoculum;

T7 – Nursery medium + *T. harzianum* + nematode inoculum;

T8 – Nursery medium + *P. fluorescens* + nematode inoculum;

T9 (Control) – Nursery medium + nematode inoculum.

For experimental purpose, one plug tray having 300 cells was selected. The tray was separated into three blocks of 90 cells each. The first block was filled with Gel-A + potting mixture, the second block with Gel-B + potting mixture, and the third block with only potting mixture (control). The three blocks were further divided into three sub-blocks each. *T. harzianum* ( $1 \times 10^8$  cfu/g) @ 5 g/150 g medium was added to one sub-block each in all the three blocks. Similarly, *P. fluorescens* ( $1 \times 10^{12}$  cells/ml) @ 5 ml/150 g medium was added to one sub-block each in the three blocks, whereas one sub-block from each block was kept as such without addition of bioagents. Seeds of tomato cv. GS-600 were sown in all the three blocks. Initially irrigation was provided after sowing the seeds and thereafter 5 ml of water was dispensed every fifth day until a month. Root knot nematode (*Meloidogyne incognita*) juveniles were introduced in all the sub-blocks @ 2 J<sub>2</sub>/cc or 20 J<sub>2</sub>/cell of plug 10 days after sowing.

The percentage of germination was recorded 10 days after sowing. At the end of the month, moisture retention of the potting mix, radicle length, plumule length and vigour index of tomato seedlings in the nursery were recorded. The final multiplication of biocontrol agents and nematode population in the potting mix were also recorded. Data were analysed using ANOVA, where percentage of moisture and percentage of germination were transformed to arcsine  $\sqrt{\%}$  in order to homogenize the variances, and

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**Table 1.** Comparison of percentage of moisture retention of different gels after different time intervals

Treatment	Percentage of moisture retention*					
	24 h	48 h	72 h	168 h	360 h	720 h
IARI gel (Gel-A)	64.1 a (53.19)	51.1 a (45.63)	36.8 a (37.35)	2.3 (8.72)	1.5 (7.04)	1.3 (6.55)
Commercial gel (Gel-B)	59.2 ab (50.30)	49.4 a (44.66)	32.1 ab (34.51)	1.3 (6.55)	1.3 (6.55)	0.7 (4.80)
Control (without gel)	56.9 b (48.97)	44.2 b (41.67)	27.5 b (31.63)	1.0 (5.74)	0.7 (4.80)	0.8 (5.13)
CD	2.96	1.83	3.53	NS	NS	NS

\*Mean of five replicates.

Figures in parentheses are arcsine transformed values.

'Means' followed by the same letter do not differ significantly according to Duncan's multiple range test at  $P = 0.05$ .

**Table 2.** Effect of hydrogels, bioagents and nematodes on germination, growth, moisture, bioagent and nematode multiplication on tomato seedlings in greenhouse nursery

Treatment	Treatment	Germination (%)*	Plumule length (cm)*	Radicle length (cm)*	Vigour index	Moisture (%)	Percentage increase in bioagents population over initial inoculum (cfu/g)	Percentage gall reduction/root over control
	<i>Pseudomonas fluorescens</i>	96.67 ab (79.53)	6.76 a	3.11 a	829	0.97 (5.65)	5900	76.7
	Without bioagent	93.33 bc (75.00)	5.53 def	2.24 efg	560	1.13 (6.02)	–	6.7
Commercial gel	<i>T. harzianum</i>	86.67 bcd (68.61)	5.80 bcde	2.69 bcd	586	2.27 (8.72)	240	73.3
	<i>P. fluorescens</i>	83.33 bcd (65.88)	6.37 abc	2.94 ab	617	0.97 (5.65)	4400	76.7
	Without bioagent	76.67 bcd (61.14)	5.03 fg	2.43 def	459	0.87 (5.35)	–	23.3
Control	<i>T. harzianum</i>	80.00 bcd (63.44)	5.33 efg	2.39 efg	495	1.90 (7.92)	–58	56.7
	<i>P. fluorescens</i>	83.33 bcd (65.88)	6.02 bcd	2.44 de	560	1.00 (5.74)	850	66.7
	Without bioagent	70.00 cd (56.79)	4.91 g	1.93 h	389	0.80 (5.13)	–	0
	CD	14.50	0.60	0.26	–	NS		

Initial nematode population: 10 J<sub>2</sub>/cc.

Initial bioagents population: *T. harzianum* –  $1 \times 10^8$  cfu/g; *P. fluorescens* –  $1 \times 10^{12}$  cfu/g.

\*Mean of three replicates.

Figures in parentheses are arcsine transformed values.

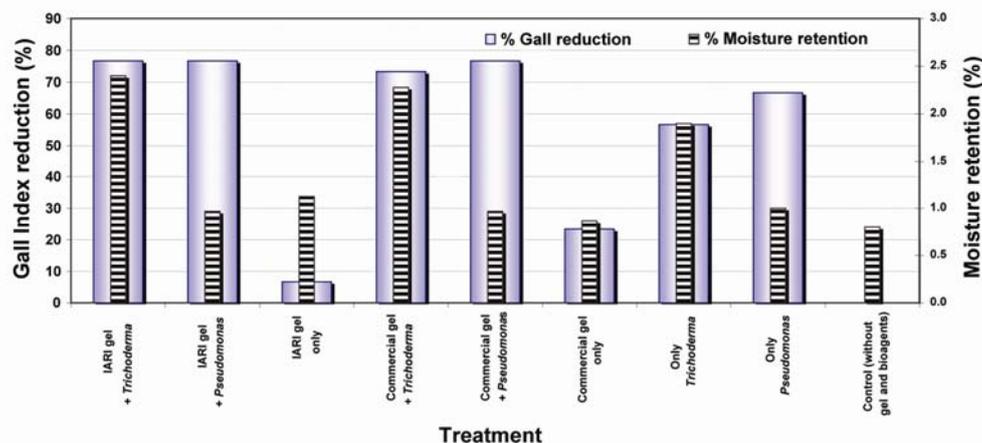
'Means' followed by the same letter do not differ significantly according to Duncan's multiple range test at  $P = 0.05$ .

the treatment means were compared using Duncan's multiple range test.

The results in the first experiment revealed that the IARI gel was more promising than the commercial gel in retaining moisture at all the time intervals studied and both the gels were superior to the control (Table 1). At 24, 48 and 72 h, the moisture retention capacity of the IARI gel was better compared to the commercial gel. However, at 168, 360 and 720 h, the moisture retention was not

at all significant. Also, perusal of the results in the second experiment indicates that the germination percentages of the tomato seedlings in general, were superior in all the treatments containing IARI gel over commercial gel and control blocks (Table 2). The IARI gel containing *T. harzianum* showed maximum germination of 100% followed by the IARI gel containing *P. fluorescens* (96.7%) and the IARI gel without bioagents (93.3%). Also, the seedlings

showed significant increase in plumule length and radicle length. The maximum plumule length was recorded in the seedlings grown in the medium comprising the IARI gel containing *P. fluorescens* (6.76 cm) followed by the IARI gel containing *T. harzianum* (6.39 cm) and commercial gel containing *P. fluorescens* (6.37 cm), whereas the maximum radicle length was recorded in seedlings grown in the medium comprising the IARI gel containing *P. fluorescens* (3.11 cm;



**Figure 1.** Effect of combination of hydrogels with bioagents on moisture retention and reduction of root-knot nematode gall index.

Table 2). The highest vigour index of seedlings (830) was observed in IARI hydrogel added treatment containing *T. harzianum*, which was on par with IARI hydrogel added treatment containing *P. fluorescens* (829), whereas the control treatment without hydrogel and bioagents had a vigour index of 389 (Table 2). The moisture retention capacities of different hydrogels + bioagents + nematodes did not show any marked significance and were at par with each other, though treatment containing the IARI gel and *T. harzianum* retained a maximum of 2.4% moisture (Table 2 and Figure 1). The results on population (cfu/g) of *P. fluorescens* over initial inoculum applied indicated 5900% increase in case of IARI gel compared to 4400% increase in commercial gel and 850% increase in control. Similarly, in case of *T. harzianum* there was 590% increase in IARI gel compared to 240% increase in commercial gel and -58% decrease in control. There was considerable decrease in the number of root knot nematode *M. incognita* galls across all bioagent added treatments, irrespective of the addition or not of the hydrogels. Percentage of gall reduction/root was observed to be superior, in the range of 56.7–76.7, in hydrogel added treatments, whereas in control treatment without gels and bioagents, 0% gall reduction/root was observed (Table 2 and Figure 1).

It is evident from the above results that in a nursery growing medium, incorporation of hydrogels has the potential to act as a reservoir of moisture. Hydrogels used over a period of time helped in conserving moisture initially, which was crucial for the germination of seeds. Later on, they were not effective in mois-

ture retention, which can be corroborated with other findings<sup>4</sup>. In the present experiment, only minimal amounts of hydrogel, on the basis of those used in earlier studies<sup>5</sup>, were exploited to accentuate the bioagent population for enhancing their efficacy to manage the nematode population. By and large, the inference gathered predicts that supplementing nursery medium with hydrogels was not only effective in enhancing the growth parameters of tomato seedlings<sup>6–8</sup>, but also contributed to the increase of the bioagent population<sup>9</sup>, which plays a key role in reducing the root knot nematode population. It is also evident from the present study that both the bioagents remained effective in reducing root knot nematode galls, though the percentage reduction was higher in nutrient medium containing hydrogels (Gel-A and Gel-B) compared to control. The IARI-developed hydrogel was marginally superior to the commercial hydrogel used, as it had better moisture retention potential. Therefore, a healthy nursery production may be advisable to supplement the potting mixture with standardized dosage of hydrogels in combination with suitable bioagents for better management of soil-borne pathogens.

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