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Table: 4, Figs : 6

Hydrogel based formulations of *Tagetes patula* root extract and $MgSO_4$ to control *Meloidogynae incognita* in cucumber

P. Adaka, A. Singh*, P. Dhiman, K.S.V. Poorna Chandika[1], S. Walia, A. Sirohi[2], V. Jain[3], M. Khandelwal[4], A. Kumar[5] and B.S. Parmar
Division of Agricultural Chemicals,
ICAR-Indian Agricultural Research Institute, New Delhi-110012, India.
E. Mail: anupama.chikara@gmail.com

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ABSTRACT

Root knot nematode (*Meloidogynae incognita*) infestation and low magnesium use efficiency are serious constraints in polyhouse cultivation of cucumber. Novel combination formulations of *Tagetes patula* root extract containing the nematicidal alpha terthienyl (α-T) and magnesium sulfate were prepared, using the biopolymeric hydrogel as carrier. The test products slowly released the nematicidal α-T with half life ($t_{1/2}$) of 22.97 and 36.87 days, in water and soil, respectively. Magnesium showed fast release till 3rd Day. Compared with commercial formulations (carbofuran, Furadan 3G®), these formulations showed significantly higher mortality of *M. incognita* population under *in vivo* and polyhouse conditions. In 3-months polyhouse study conducted on cucumber crop during 2011-12, the application of formulations containing Mg alone or Mg in combination with root extract significantly increased the crop yield and showed 83% correlation with the magnesium content of leaves. Besides the morphological and physiological parameters of the crop were also improved.

KEYWORDS: Carbofuran, cucumber, *Cucumis sativus*, formulations, furadan, hydrogel, magnesium, *Meloidogyne incognita*, nematicide, root extract, *Tagetes patula*.

INTRODUCTION

Cucumber (*Cucumis sativus* L. family cucurbitaceae) is important economical crop, grown in the fields and under protected conditions. Protected cultivation aims to produce high value horticultural crops in modified environment (33). Root-knot nematode, (*Meloidogyne incognita*) and magnesium nutrition have been identified as the key constraints in vegetable production. In India, the problem of nematodes is more severe in protected cultivation than in field cultivation (19). *M. incognita* nematode causes major yield losses in vegetable crops (21,24). Repeated application of carbofuran nematicide is recommended for its control (25). However, it has serious environmental issues owing to its mobility in soils (16). Marigold (*Tagetes* sp.) plant is an effective and proven bio-pesticidal option against plant-parasitic nematodes (25,29,31). *T. erecta* and *T. patula* are used in medicinal, biopesticidal and nutraceutical applications. The flowers are source of

*Corresponding authors, [1]Indian Institute of Oilseeds Research, Hyderabad, India, [2]Division of Nematology, Indian Agricultural Research Institute, New Delhi, India, [3]Indian Council of Agricultural research, New Delhi, [4]Division of Design of Experiments, Indian Agricultural Statistical Research Institute, New Delhi, India.

essential oil, food colorants and antioxidant lutein. Marigold yields highly nematicidal compound alpha-terthienyl (α-T, Fig. 1), from its roots (8,10). The cucumber responds well to magnesium, but its application is ignored in fertilizer recommendations. Magnesium is the central element of chlorophyll molecule (14), carrier of phosphorus in the plant, an enzyme activator (phosphorylation and dephosphorylation), a constituent of many enzymes and is essential for plant functions like photosynthesis (11).

Owing to low use efficiencies of inputs and environmental pollution, carrier based controlled release (CR) formulation technology of agro-inputs especially pesticides and nutrients is of current interest (2,23). Water insoluble hydrogels with loosely cross linked three dimensional network structures and high fluid absorption characteristics are finding applications as carriers in targeted delivery of water, drugs, pesticides and nutrients (1,2,4,5,6,20,23,27,29,32,33). Polyelectrolytic superabsorbents exhibit swelling response to the external pH and ionic strength, which serve as switches for controlled release of a.i. (active ingredient) from the matrix (7,18,22). In this study, a cellulosic cross-linked polyacrylate hydrogel was used as carrier to develop the formulations containing root extract of *Tagetes* spp. and $MgSO_4$. This study aimed to (i) assess the potential of novel approach of controlled delivery of nutrients and the biopesticide through hydrogel in rhizosphere under polyhouse conditions and (ii) determine the effects of different hydrogel based formulations on the population of *M. incognita* and yield of cucumber crop raised under protected conditions.

MATERIALS AND METHODS

Laboratory grade solvents were distilled before use. HPLC grade acetonitrile, methanol and water were used for HPLC analysis and other analytical work. Carbofuran (3% encapsulated granule) was procured from FMC India (P) Ltd., District Kanchipuram, Tamil Nadu. Alpha terthienyl (α-T, $C_{12}H_8S_3$, 99%, syn. Terthiophene, 2, 2':5', 2''-Terthiophene, Fig. 1, pale yellow solid, m.p. 93-95^{0}C, b.p. 160^{0}C, insoluble in water) was procured from Sigma-Aldrich. Stock solution of α-T (1000 ppm) was prepared in HPLC grade acetonitrile. Working standard solutions of 2,5,10,20,25 and 200 ppm were prepared from the stock solution by serial dilution and stored at 5^{0}C till further use. Laboratory grade $MgSO_4$ was purchased from Merck Specialties (P) Ltd. New Delhi, India. Cellulosic anionic polyacrylate hydrogel (Kauvery Hydrogel, off white amorphous powder, 60-100 mesh, pH in swollen state 7.0 at 30^{0}C in distilled water, and equilibrium swelling ratio 500 in distilled water), used as carrier in test formulations was purchased from Carborandum Universal. Ltd., Chennai, India.

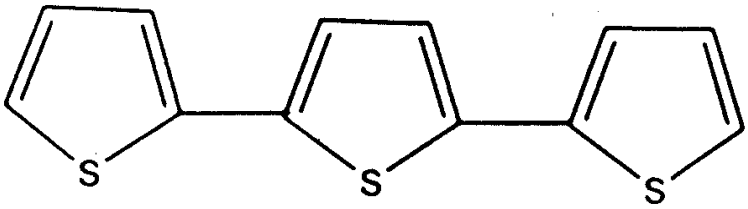

Figure 1. Chemical structure of 2,2':5',2"-TERTHIOPHENE (α-T)

Sandy loam soil (0-15 cm, 2mm; sand 66%, silt 9%, clay 25%; organic carbon 0.30%; pH 6.3 at 1:1.25 soil: water; EC 0.15 dS m^{-1}) and soil less medium (cocopeat, perlite and vermiculite in the ratio of 4:1:1 on volume basis) was collected from institute's Farm. Second stage juveniles (J2) of *Meloidogyne incognita* were obtained from a pure culture previously initiated by egg masses and propagated on tomato (*Lycopersicon esculentum*) in a glasshouse.

Ninety days old plants of *Tagetes patula* (var. Arpita) were collected from the Division of Floriculture of our Institute. Roots were washed, excised from shoots and dried at 45ºC till constant weight. Dried roots (400 g) were chopped, ground in a laboratory blender and Soxhlet extracted, first with hexane followed by ethyl acetate and methanol, for 8 h each. Solvents were removed in a rotavapor under reduced pressure, extracts collected, dried and stored at 5^{O}C in a refrigerator.

Instruments. Estimation of α-T was done in High Performance Liquid Chromatograph (HPLC) fitted with solvent delivery module Varian Prostar model 240 as chromatographic pump, Lichrospher (250-4, 5 µm) RP 18 column and UV detector (chromatographic conditions: mobile phase, methanol-acetonitrile-water (40:40:20, v/v); flow rate, 1ml min^{-1}; absorbance at 350 nm; α-T retention time, 14.32 minutes). The analysis showed linearity in the range of 1-25 µg mL^{-1}. Magnesium was estimated using Flame atomic absorption spectrometer of Electronic Corporation of India Ltd. model AAS 4141 fitted with magnesium lamp and connected to acetylene gas source was used for the estimation of magnesium at 284.6 nm wave length.

Preparation of formulations. Hexane, ethyl acetate and methanol extracts of roots were analyzed by HPLC and it was found that only hexane extract of the root showed the presence of α -T (34 mg g^{-1} of root extract). Formulations were prepared separately for the *in-vitro* and *in-vivo* (polyhouse bioefficacy) evaluation. For *in-vitro* bioefficacy evaluation, the test formulations were prepared by impregnation technique (Table 1). Dry hydrogel powder (0.5 g) was swollen in 10 mL emulsion (5% emulsifier, Triton-X 100 and Span 80 in 3:2 ratios) containing root extracts of different concentrations with continuous stirring at 25ºC for 1 h. The gel mass formed was oven dried at 45^{0}C to constant weight, yielding light brown colored brittle mass which was ground to particles of 100 -120 mesh size. Impregnation of magnesium sulfate alone in dry hydrogel powder (0.5 g) was done by allowing to swell in emulsified magnesium sulfate solution (10%, 10 mL) for overnight at 45^{0}C with stirring and then oven dried, yielding dry mass which was ground to particles of 100 -120 mesh size. Impregnation of root extract along with magnesium sulfate in gel mass was achieved by adding dry root extract impregnated gel mass in aqueous magnesium sulfate solution (10%, 10 mL) and allowed to swell for overnight at 45^{0}C with stirring, oven dried to get dry mass which was ground to 100-120 mesh size.

For *in vivo* bioefficacy evaluation, 17-test compositions were prepared using two approaches, impregnation and adsorption **(**Table 2**)**. In impregnation method, root extract in hexane (2 ml) and 0.4 g of magnesium sulfate were impregnated in 4 g of dry gel in stepwise manner. In adsorption method, dry hydrogel powder (4 g, 100-120 mesh) was treated with 10 mL hexane containing required quantity of root extract in a 100 ml beaker with continuous mechanical stirring for 1 h at 25ºC. The gel mass was dried at 45^{0}C. This

extract was enriched by mixing with dry hydrogel powdered magnesium sulfate (8 g) in a pestle and mortar at room temperature. We got the free flowing powder containing the root extract and $MgSO_4$. These compositions were used as such in the pot study.

Table 1. Composition of test products prepared for *in-vitro* bioassay

Test product Code	Root extract (ppm)	α-T (ppm)	$MgSO_4$ (% w/w carrier)
IM- 125	125	85	-
IM- 64	64	43.52	-
IM- 32	32	21.76	-
IM- 16	16	10.88	-
IM-8	8	5.44	-
IM- 125-Mg	125	85	10
IM- 64-Mg	64	43.52	10
IM- 32-Mg	32	21.76	10
IM- 16-Mg	16	10.88	10
IM-8-Mg	8	5.44	10
IM-Mg	-	-	10

To estimate effective loading of α –T in deveoped formulations, 1.0 g of formulation was extracted five times with 10 mL hexane. To ensure maximum extraction of bioactive compound, the vessel content were sonicated for 1 h. The concentrated extract was dissolved in 1 mL HPLC grade acetonitrile and estimated for α-T by HPLC. The recovery of α-T from dry formulation was calculated as under:

$$\text{Recovery of } \alpha\text{-T (\%)} = \frac{\text{Amount of magnesium detected in dry formulation}}{\text{Amount of magnesium loaded in formulation}} \times 100$$

Recovery of α-T from different test formulations was: AD-192 (98.67%), AD-384 (98.60%), IM-192 (97.73%) and IM-384 (97.04%).

Magnesium content loaded in the test formulations (T-Mg-F and T-Mg-H) was evaluated by treating the dry formulation (1 g) with 100 mL water, shaking vigorously overnight, centrifugation at 20000 rpm and finally collecting the supernatant. The process was repeated twice. All extracts were combined, filtered through Whatman filter paper No.42 and magnesium was estimated using Atomic Absorption Spectrophotometry at 284.3 nm. The recovery of magnesium from dry formulation was calculated as under:

$$\text{Recovery of Magnesium (\%)} = \frac{\text{Amount of magnesium detected in dry formulation}}{\text{Amount of magnesium loaded in formulation}} \times 100$$

Recovery of Magnesium from T-Mg-F and T-Mg-H was 93.8 and 95%, respectively.

Table 2. Test products prepared with Tagetes extracts and $MgSO_4$ for field bioefficacy evaluation

S. No.	Test product Code	Root extract (% w/w carrier)	α-T (% w/w carrier)	$MgSO_4$ content (g/pot dry formulation)	Method of incorporation
1	AD- 0.384%	0.384	0.013	0.0	Adsorption (T. extract)
2	AD- 0.786%	0.786	0.016	0.0	Adsorption (T. extract)
3	AD- 0.384%- Mg(F)	0.384	0.013	Full dose (4g/pot)	Adsorption (T. extract) and blend ($MgSO_4$)
4	AD- 0.384%-Mg(H)	0.384	0.013	Half dose (2g/pot)	Adsorption (T. extract) and blend ($MgSO_4$)
5	AD- 0.786%-Mg(F)	0.786	0.016	Full dose (4g/pot)	Adsorption(T. extract) and blend ($MgSO_4$)
6	AD- 0.786%-Mg(H)	0.786	0.016	Half dose (2g/pot)	Adsorption (T. extract) and blend ($MgSO_4$)
7	IM- 0.384%	0.384	0.013	0.0	Impregnation (T. extract)
8	IM- 0.786%	0.786	0.016	0.0	Impregnation (T. extract)
9	IM- 0.384%-Mg(F)	0.384	0.013	Full dose (4g/pot)	Impregnation (T. extract) and $MgSO_4$
10	IM- 0.384%-Mg(H)	0.384	0.013	Half dose (2g/pot)	Impregnation (T. extract) and $MgSO_4$
11	IM- 0.786%-Mg(F)	0.786	0.016	Full dose (4g/pot)	Impregnation (T. extract) and $MgSO_4$
12	IM- 0.786%-Mg(H)	0.786	0.016	Half dose (2g/pot)	Impregnation (T. extract) and $MgSO_4$
13	IM-Mg(H)	Nil	Nil	Half dose (2g/pot)	Impregnation ($MgSO_4$)
14	IM-Mg(F)	Nil	Nil	Full dose (4g/pot)	Impregnation ($MgSO_4$)
15	AD-Mg(H)	Nil	Nil	Half dose (2g/pot)	Blend ($MgSO_4$)
16	AD-Mg(F)	Nil	Nil	Full dose (4g/pot)	Blend ($MgSO_4$)

H : Half recommended dose of $MgSO_4$=2.0 g per plot), F: Full recommended dose of $MgSO_4$ = 4.0 g per plot; Recommended rate of Mg @ 6Kg/ Ha. T. extract : Tagetes extract.Adsorption: Tagetes extract adsorbed on the surface of hydrogel particles, Blend: Magnesium sulphate powder was physically blended with tagetes extract and hydrogel powder, Impregnation: Tagetes extract and magnesium sulphate impregnated in the hydrogel matrix *in situ*. (AD: Adsorption and blend and IM: Impregnation).

Release kinetics. The kinetics of α-T release was studied in water. Test formulation AD-0.384% (0.5 g) was taken in nylon bag (200 mesh; 5 × 4 cm) in triplicate. Each bag was suspended in 130 ml distilled water contained in a beaker. At each destructive sampling (0, 1, 3, 7, 14, 21, 30, 45 and 60 days), the nylon bags were removed and the water was analyzed for α-T by HPLC. To study the release of magnesium from test formulation, T-Mg-F (0.01 g) was taken in parchment capsules (5× 5 cm) and placed in 25 mL water in stoppered conical flasks. Capsules were taken out periodically (0, 1, 3, 7, 14, 21, 30 and 45 days) and the filtrate was analyzed by Atomic Absorption Spectrophotometer. Release kinetics was studied using PROC REG & PROC RSREG (SAS 9.2).

The release data were fitted to semi empirical equation (20) to get diffusion exponents.

$$M_t/M_0 = Kt^n \quad (1)$$

Where, M_t / M_o : Fraction of active ingredient released at time t. K : Constant that incorporates characteristics (porosity, tortuosity) of the macro molecular network system and the active ingredients, and n : Diffusion parameter indicate the transport mechanism.

***In-vitro* bioefficacy.** Twenty-three treatments comprising of different combinations and four controls namely magnesium sulfate (full rate) alone, carbofuran (Furadon 3G®) at full and half rates and absolute control were evaluated in triplicate. Description of test products is given in Table 1. Finely powdered test formulation (0.5% on soil weight basis) was added to autoclaved soil (5 g) in a Petri dish and mixed thoroughly. Nematode suspension (1 mL containing 50 J2 in water) was added to it. Soil moisture was monitored and maintained gravimetrically. Nematode counts were taken at 24 and 48 h. The nematode count was done as per Whitehead and Hemming (31) with minor modifications. Live nematodes were counted under stereoscopic binocular microscope. The mortality (%) was calculated. LC_{50} values (ppm) were found using PROC–PROBIT (SAS 9.2) programme.

***In-vivo* bioefficacy**. Farm soil from Centre of Protected Cultivation Technology, Indian Agricultural Research Institute (IARI), New Delhi was autoclaved, mixed with well decomposed autoclaved farm yard manure and filled into pots (15 cm diameter) @ 450 g soil / pot. Test formulations including carbofuran (Furadon 3G®) were thoroughly mixed with the soil. Treatment details are given in Table 6. Ten seeds of cucumber (var. Aviva) were sown in each pot and 10 mL of suspension containing 900 J2 nematodes was added per pot. Modifications in the timing and method of addition of nematode suspension was done to simulate actual field situation. Pots were kept in polyhouse and watered regularly. After 30 days, the seedlings along with the roots were carefully removed from the soil, and soil washed by Cobb decanting and sieving method (5). Nematodes were collected in distilled water (28). Numbers of live and dead nematodes were counted.

Polyhouse evaluation. Field study was conducted during 2011-12 in naturally ventilated polyhouse, Centre for Protected Cultivation Technology, IARI. Twenty nine treatments including 16 test compositions (Table 2) were replicated three times in completely randomized block design. Cucumber (var. Aviva) seedlings (35d old) were transplanted in 2m × 1 m plots at 15 cm spacing in the polyhouse. Test Formulations were applied in the root zone during the transplanting of seedlings. The effect of formulations was determined in terms of morphological, physiological and yield response of the plants. At 30 days after transplanting, leaf area (cm^2) was measured using LICOR-3000 leaf area meter. Number of flowers per plant (Mean of 10 plants per treatment) was also recorded on the same day. Magnesium (%) in leaf tissue (13), chlorophyll content in leaves (11) and yield were also determined. Total yield of all pickings of the replicates of each treatment was recorded. The data were analyzed statistically using PROC GLM (SAS 9.2).

Statistical analysis. The data was analyzed using SAS PROC NLIN and PROC REG.

RESULTS AND DISCUSSION

Kinetics of α-T and magnesium release in water

Periodic release of α-T from a representative formulation AD-0.384% in water is shown in Fig. 2. Release of α-T from the formulation increased till 14^{th} day and then become gradual up to 60^{th} day. The values of release rate (K) and diffusion coefficient (*n*) obtained from α-T released in water are presented in Table 3. The value of diffusion exponent in water was 0.75, it suggests anomalous transport as the operating release mechanism. The half release time ($t_{1/2}$) of α-T in water was 22.97 days, clearly confirming that the test formulations behaved as slow release products. Low solubility of α-T in water

coupled with crosslinked network structure of hydrogel carrier contributed to this type of release behavior. Release behavior of magnesium from the test formulation T-Mg-F in water showed maximum release on 3rd day followed by consistent decrease till 45th day (Fig. 2). However, even on the 45th day, the Mg content in water was above 100 ppm. The values of K and *n* obtained from magnesium released in water were 0.49 and 0.146 (Table 3). The half release time of magnesium in water was 1.104 days, indicating that its release in water from the combination formulation was much faster than that α-T.

Table 3. Constants and $t_{1/2}$ values derived from fitting of empirical equation $M_t / M_o = kt^n$ to release data of α-T in water and soil from test formulation AD-0.384% and magnesium in water from test formulation Mg-F

Formulations	Medium	K	n	r^2	Prob.> F	$t_{½}$ (Day)
AD- 0.384%	Water	0.04	0.75	0.97	<.0001	22.97
AD- 0.384%	Soil	0.03	0.75	0.94	<0.0001	36.87
T-Mg-F	Water	0.49	0.14	0.91	0.0002	1.10

K : Constant related to structural and geometric properties of the delivery system, n : Diffusional parameter to indicate the transport mechanism, r^2 : Correlation coefficient

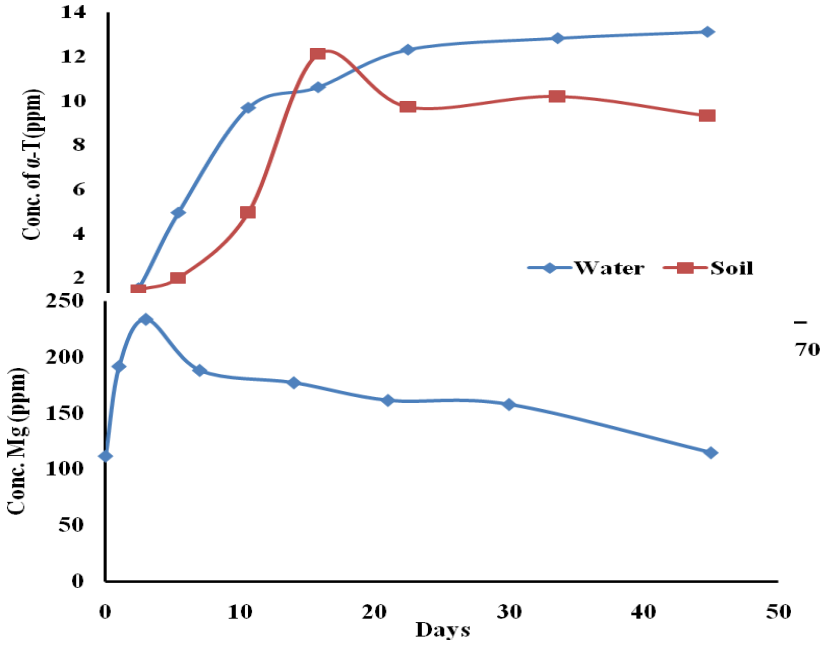

Figure 2. Cumulative release of (A) Tagetes root extract from test formulation AD- 0.384% in water and soil and (B) magnesium sulphate from test formulation T-Mg-F in water. Error bars represent the standard deviation of three replicates

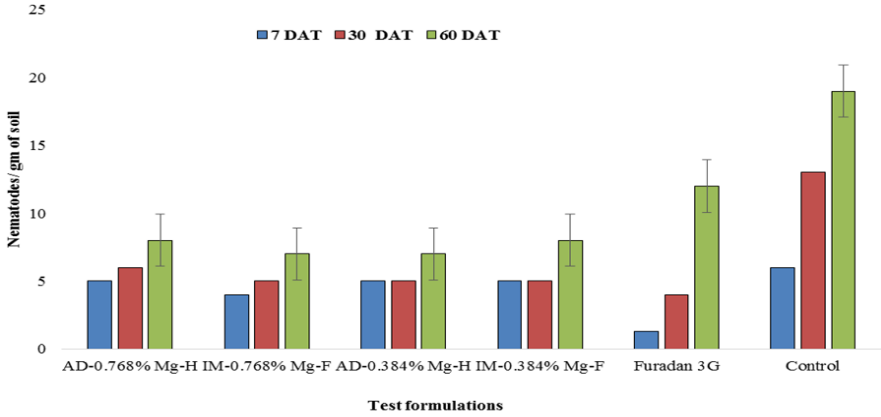

Figure 3. Effects of treatments on population of *M. incognita* in polyhouse soil at different days after transplanting

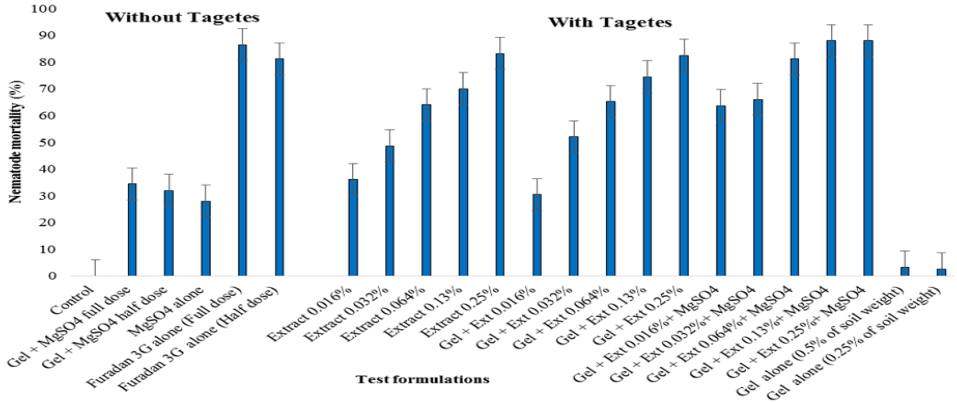

Figure 4. Effects of test formulations on *in vitro* mortality (%) of *M. incognita*
5 g soil was added in each petri dish, dry hydrogel@ 0.025 g and 0.05 g respectively was added in gel alone treatments (0.25% and 0.5% respectively); $MgSO_4$ was added @ 0..005 g (Half dose) and 0.01 g (full dose); Furadan full dose (10 Kg a.i./ ha) and half dose (5 Kg a.i./ha)

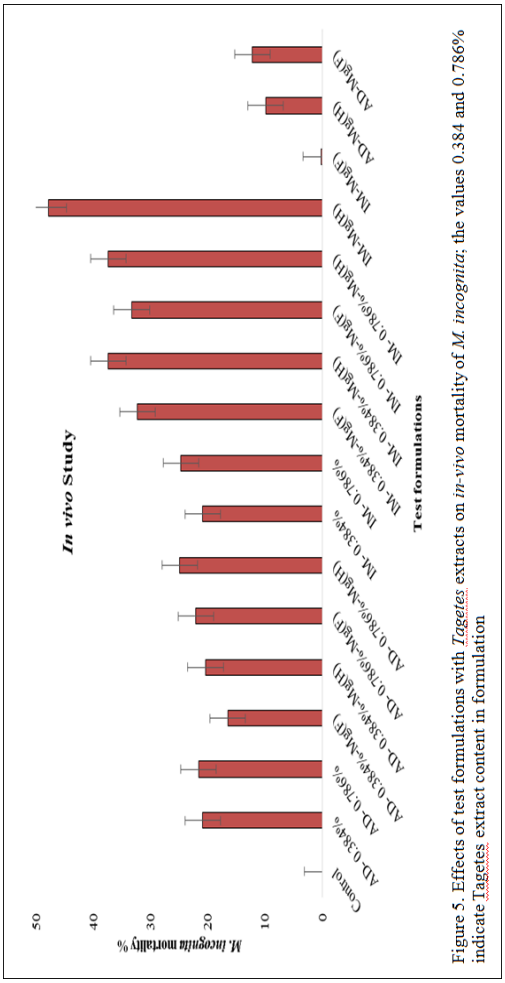

Figure 5. Effects of test formulations with *Tagetes* extracts on *in-vivo* mortality of *M. incognita*; the values 0.384 and 0.786% indicate Tagetes extract content in formulation

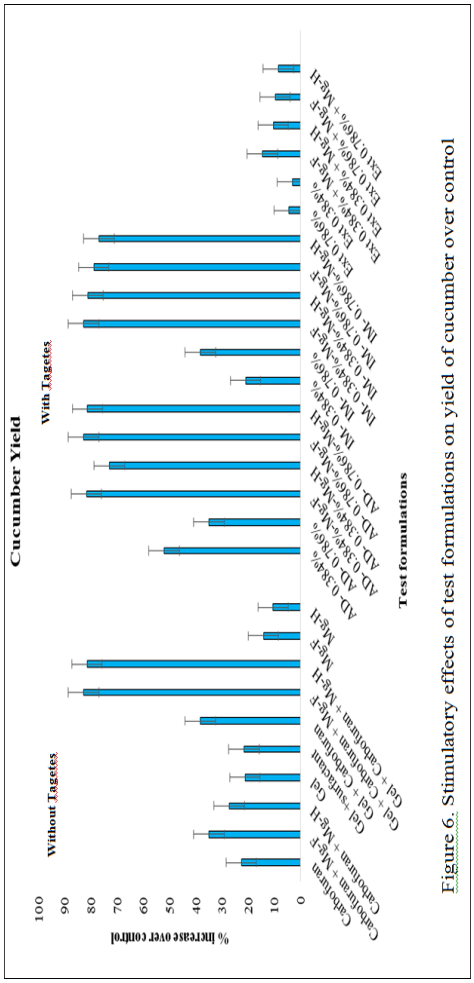

Figure 6. Stimulatory effects of test formulations on yield of cucumber over control

Nematicidal activity in polyhouse soil. On the day of transplanting, the mean nematode population in polyhouse soil was 6 nematodes per g soil. Control registered increase in population to 13 and 19 nematodes per g soil on 30^{th} and 60^{th} d after transplanting (Fig. 3). Furadan 3G® application controlled 69 % nematodes over control till 30^{th} day, but on 60^{th} day control dropped to 37%. Notably, the *Tagetes* roots extract and magnesium based test formulations, continued to control the nematodes population (4-8 nematodes per g soil) throughout the study period, registering an average 42% consistent inhibition over control. On 60^{th} day, all test products showed 65% control of nematodes than Furadan (37% control). This may be owing to the slow release characteristics of the test product ($t_{1/2}$ 36.87 days) and the release coinciding with the 30-days life cycle of *M. incognita*. *In-vivo* evaluation of the nematicidal activity was done to simulate farmers' field conditions, where all effects including natural mortality of nematodes due to high temperature, non-availability of plant roots etc. operate. These factors may be responsible for decline in the number of nematodes from initially applied 900 J2s to 393 J2s, which was also seen in other treatments. Formulations based on extract alone showed relatively lesser mortality of nematodes. Role of magnesium in enhancing the bioefficacy of extracts may be due to desiccation caused by the presence of magnesium sulfate in water surrounding rhizosphere leading to high osmotic potential and the resultant exosmosis from the body of the pest.

Bioefficacy

***In vitro* and *in vivo* nematicidal activity of test products:** *In-vitro* nematicidal activity expressed as % mortality of J2 larvae in various treatments is presented in Fig. 4. Hydrogel formulations containing 0.13 or 0.25% extract with full dose of $MgSO_4$ exhibited maximum mortality (88%). Thus when $MgSO_4$ was combined with extract in gel formulations, significantly high mortality was observed in all test concentrations. Combination formulations with lower concentrations of extract (0.032% and 0.064%) were as effective as the higher concentrations without $MgSO_4$ in gel formulations. Combined formulations up to 0.064% extract were statistically comparable with Furadan 3G.

During *in-vivo* bioeffcicacy evaluation of test formulations for calculating mortality (%) of J2s, live nematode population in control was assumed as 100%. Carbofuran was most effective against *M. incognita* registering 47.83% mortality of juveniles (Fig. 5). The combination test products AD- 0.768% -Mg-F and IM- 0.768% -Mg-F caused 37.40 % mortality. Corresponding treatments containing half equivalent of $MgSO_4$ *viz.* AD- 0.768% -Mg-H, IM- 0.768% -Mg-H also caused 32.31 and 33.33% mortality. Hydrogel alone did not show any mortality and behaved as control.

Polyhouse evaluation: The treatments containing both extract and magnesium gave maximum yield than control (Fig. 6.) There was no significant difference in the yield of cucumber with half dose of magnesium (8.5-81.4 %) and full its dose (9.8-82.9 %). The hydrogel + Furadon 3G + magnesium yielded 82.9% higher over control. In general the hydrogel as carrier stimulated the yields (38.3-82.9 % yield increase) over the non-hydrogel treatments. Amongst the hydrogel based treatments, those containing magnesium (irrespective of full or half of recommended dose) increased the yields (9.8 to 82.9 %) over those without magnesium (10.6 to 14. 3 %). Treatments comprising combined application of tagetes roots extracts with magnesium showed higher yield (8.5 to 14.6 %) over control

than those involving the roots extract alone (3.2 to 4.5 %). Here also, the relative yield increase was higher in hydrogel based treatments (21.1 to 52.2 % with extract alone and 73.1 to 82.9 % in extract + magnesium). The better performance of gel based treatments is attributed to the controlled release and hydrophilic properties of the hydrogel as carrier.

Table 4. Effects of test formulations on morphological and physiological parameters and yield of cucumber

Treat No.	Treatments	Leaf Mg (%)	Total chlorophyll (mg/FW)	Leaf area (cm^2)	No. of flowers / plant	Cucumber yield (Tones/ha)
			Without Tagetes extract			
1	Control	0.33 ^{f}	3.27 ^{f}	308.77 ^{fg}	3.12 ^{l}	20.59 ^{h}
2	Carbofuran	0.33 ^{f}	3.08 ^{f}	309.14 ^{f}	5.15 ^{fghi}	30.36 ^{de}
3	Carbofuran + Mg-F	0.39 ^{bcd}	5.55 ^{cde}	351.22 ^{d}	5.85 ^{cdef}	34.00 ^{c}
4	Carbofuran + Mg-H	0.39 ^{bcd}	5.10 ^{e}	352.84 ^{d}	5.05 ^{fghij}	32.08 ^{cd}
5	Gel	0.33 ^{f}	3.23 ^{f}	336.23 ^{e}	4.51 ^{hijk}	30.55 ^{de}
6	Gel +Surfactant	0.33 $_{f}$	3.24 ^{f}	337.53 ^{e}	4.36 ^{ijk}	30.66 ^{de}
7	Gel + Carbofuran	0.33 ^{f}	3.22 ^{f}	394.06 ^{c}	5.68 ^{def}	34.85 ^{c}
8	Gel + Carbofuran + Mg-F	0.43 ^{abc}	6.00 ^{bcd}	337.07 ^{e}	7.02 ^{ab}	46.07 ^{a}
9	Gel + Carbofuran + Mg-H	0.41 ^{abcd}	5.35 ^{e}	336.23 ^{e}	6.55 ^{bcd}	45.75 ^{a}
10	Mg-F	0.39 $_{bcd}$	5.55 ^{cde}	352.73 ^{d}	5.25 ^{efgh}	28.80 ^{efg}
11	Mg-H	0.38 $_{de}$	5.10 ^{e}	351.45 ^{d}	4.69 ^{ghijk}	27.85 ^{efgh}
			Tagetes extract			
12	AD- 0.384	0.33 $_{k}$	3.27 ^{f}	336.05 ^{e}	5.21 ^{efgh}	38.35 ^{b}
13	AD- 0.786	0.33 $_{f}$	3.06 ^{f}	336.08 ^{e}	4.28 ^{jk}	34.02 ^{c}
14	AD- 0.384-Mg-F	0.44 ^{a}	6.20 ^{abc}	404.30 ^{ab}	4.28 ^{jk}	45.8 ^{a}
15	AD- 0.384-Mg-H	0.41 ^{abcd}	5.01 ^{e}	408.07 ^{a}	3.39 ^{l}	43.6 ^{a}
16	AD- 0.786-Mg-F	0.43 $_{ab}$	6.74 ^{a}	411.54 ^{a}	6.58 ^{abc}	46.08 ^{a}
17	AD- 0.786-Mg-H	0.41 $_{abcd}$	5.01 ^{e}	394.05 ^{c}	6.47 ^{bcd}	45.7 ^{a}
18	IM- 0.384	0.34 $_{ef}$	3.26 ^{f}	337.04 ^{e}	5.25 ^{efgh}	30.5 ^{de}
19	IM- 0.786	0.34 $_{ef}$	3.10 ^{f}	336.21 ^{e}	6.45 ^{bcd}	34.83 ^{c}
20	IM- 0.384-Mg-F	0.43 $_{abc}$	6.16 ^{abcd}	392.27 ^{e}	6.66 ^{abc}	46.06 ^{a}
21	IM- 0.384-Mg-H	0.41 $_{abc}$	5.05 ^{e}	393.09 ^{c}	5.15 ^{fghi}	45.66 ^{a}
22	IM- 0.786-Mg-F	0.43 $_{abc}$	6.37 ^{ab}	396.42 ^{bc}	7.33 ^{a}	45.09 ^{a}
23	IM- 0.786-Mg-H	0.41 $_{abcd}$	5.53 ^{de}	395.40 ^{c}	5.25 ^{efgh}	44.58 ^{a}
24	Ext 0.786	0.33 $_{f}$	3.34 ^{f}	300.79 ^{g}	3.22 ^{l}	26.33 ^{gh}
25	Ext 0.384	0.33 $_{f}$	2.92 ^{f}	313.61 ^{f}	3.33 ^{l}	26 ^{gh}
26	Ext 0.384 + Mg-F	0.38 $_{de}$	5.55 ^{cde}	350.91 ^{d}	5.33 ^{efg}	28.88 ^{efg}
27	Ext 0.384 + Mg-H	0.39 $_{cd}$	5.10 ^{e}	349.90 ^{d}	5.11 ^{fghi}	27.88 ^{efgh}
28	Ext 0.786 + Mg-F	0.38 $_{de}$	5.53 ^{de}	351.08 ^{d}	6.00 ^{cde}	27.66 ^{efgh}
29	Ext 0.786 + Mg-H	0.39 $_{cd}$	5.06 ^{e}	352.34 ^{d}	4.22 ^{k}	27.33 ^{fgh}
	CV	1.68	4.49	0.712	4.83	2.804

*Means with in a column followed by same letters are not significantly different (P<0.0001)
Mg-F (Magnesium full dose), Mg-H (Magnesium half dose), AD (Tagetes extract adsorbed), IM (Tagetes extract impregnated), Ext (Only Tagetes crude extract). FW: Fresh weight

Morphological and physiological parameters. All treatments containing magnesium showed higher number of flowers per plant than absolute control and control without magnesium (Table 4). In yield parameters, the effect was more pronounced in hydrogel based treatments. Application of extract based formulations/extract alone or Furadan 3G did not exhibit any effect. Method of mixing the Mg and extract in the carrier also did not show any inhibitory or stimulatory effect. Similar trend was in cucumber plants leaf area.

The application of $MgSO_4$ increased the magnesium content in leaves of cucumber plants (Table 4). The magnesium content in leaves from plants treated with hydrogel based magnesium amendments increased the magnesium content (0.41 to 0.43 % higher over control) in leaves of cucumber than in non-hydrogel based magnesium treatments (0.38 to 0.39 % higher over control). The application of magnesium dose did not influence the final magnesium content in leaves. Total chlorophyll content in leaves also showed similar pattern. Role of magnesium in enhancing the rate of photosynthesis through increase in chlorophyll content of leaves is well established (15). Hence he increased Mg (0.43 % higher than control) in the tissues can be correlated with the higher levels of chlorophyll (6.74 mg/fresh wt). The hydrogel used as carrier in this study also enhanced the moisture availability in soil (27,29). Incorporation of magnesium in hydrogel resulted in enhanced availability and uptake of magnesium by the growing roots of cucumber as reflected in significant increase in yield than control (Fig. 6). Correlation was 83% between leaf magnesium content and yield and 85.3% between magnesium content and chlorophyll content of leaves. Overall enhancement in crop health was reflected in increased dry matter and enhanced leaf area with 56% correlation between magnesium content and leaf area. Enhanced water and overall nutrient use efficiency induced by the presence of hydrogel in rhizosphere zone and suppressed nematicidal activity appear to be the key factors in this study in improving yields. While conventional application of magnesium showed enhancement in yield of cucumber, statistically similar yields were obtained in formulations containing half dose equivalent of the element. This could be explained in terms of calculated enhanced period of availability of magnesium in the carrier matrix in root zone as compared to the conventionally applications. This finding also suggests the potential of the test products in reducing the recommended dose of secondary nutrient application without decreasing the crop productivity.

CONCLUSIONS

This study showed a unique approach of integrated pest and nutrients management. Hydrogel carrier based combined controlled release formulations of root extract of *T. patula* and $MgSO_4$ proved superior to other management practices by suppression of *M. ingonita* population and the improved plant health and yield. Not only this, half dose of magnesium sulfate + hydrogel improved the plant health and yield equivalent to the full dose. Further studies are required to establish the twin potential of prepared formulations under different soil and climate conditions.

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