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Development of Controlled Release Formulations of Carbofuran and Evaluation of Their Efficacy against *Meloidogyne incognita*

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Controlled release (CR) formulations of the insecti-nematicide carbofuran have been prepared using commercially available rosin, sodium carboxymethylcellulose and sodium carboxymethylcellulose with clay (bentonite, kaolinite, and Fuller's earth). The kinetics of carbofuran release in soil from the different formulations were studied in comparison with that of the commercially available granules (3G). Release from the commercial formulation was faster than with the new CR formulations. Addition of clay in the biodegradable polymer matrix reduced the rate of release. The diffusion exponent (n value) of carbofuran in soil ranged from 0.462 to 0.740 in the tested formulations. The half-release ($t_{1/2}$) values ranged between 4.79 and 25.11 days, and the period of optimum availability (POA) of carbofuran ranged from 15.10 to 43.97 days. The mean EC_{50} of the commercial formulation against *Meloidogyne incognita* was quite high as compared to those of CR formulations. The effective duration (t_e) of carbofuran from the CR and commercial formulations was predicted by fitting the mean EC_{50} values of test formulations in the model $(M_\infty - M_e)/M_\infty = K_d t_e$. It was 0.7 day in commercial 3G in comparison with 17.8 days for CMC−bentonite. The bioassay studies revealed that with the rosin-yellow polymer, the dose of carbofuran could be reduced to half of its recommended dose for nematode control. Overall, a comparison of CR formulations with the commercial one showed an earlier degradation of carbofuran in the latter and relatively prolonged activity in the former.

KEYWORDS: Controlled release; carbofuran; insecti-nematicide; rosin, sodium carboxymethylcellulose; clay; bentonite; kaolinite; Fuller's earth; nematicidal activity; *Meloidogyne incognita*

INTRODUCTION

Controlled-release (CR) technology for pesticides has received increasing attention due to a growing awareness of the undesirable environmental effects of pesticides as observed from their application through conventional formulations to attain levels required for effective pest control (*1*). The use of CR formulations regulates the supply of the active ingredient (ai) at the required rate for pest control, thus reducing the amount of chemical introduced in the environment and protecting it from its deleterious effects. These permit safer, efficient, and economical crop protection, reduce phytotoxicity, degradation, leaching, and chemical load in the environment, and enable convenient handling and distribution as well as an extended release period of chemicals. The beneficial effects of CR formulations are highlighted in several publications (*2*−*5*). Due to the various advantages, numerous examples are available in the literature wherein such products have been effectively employed to combat pests (*6*−*10*).

Carbofuran [2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate] is a systemic insecti-nematicide for soil and foliar treatments. It has been identified as a potential leacher by using the groundwater ubiquity scores (GUS) modeling technique (*11*). Its high acute toxicity, LD_{50} (rat) $= 8$ mg kg^{-1} (*12*), is of environmental concern.

To achieve control of nematodes, it is necessary to maintain a regulated supply of an appropriate concentration of chemical in the plant rhizosphere. To counter environmental losses and maintain the concentration above the minimum threshold of activity, application of an excessive amount of conventional formulation of carbofuran is required. An increase in application rate, however, results in an increase in the potential adverse impact on the environment. The excessive quantities increase the runoff or leaching and thus the pollution of surface or ground water (*13*). The use of an alginate-based CR formulation has been reported to reduce carbofuran movement compared to the technical product in soil (*5*). However, no information on its use in pest control has been reported. Use of bentonite and humic acid as modifying agents in alginate-based CR formulations of imidacloprid is reported (*14*). Lignin-based CR formulations of imidacloprid and 2,4-D are also reported in refs *4* and *15*, respectively. The persistence of thiobencarb in the

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alginate−kaolin-based CR formulation was prolonged in water and soil (*16*). The use of bentonite clay in regulating the release of carbofuran is reported in ref *17*.

Rosin and rosin-based polymers have diversified drug delivery applications achieving sustained/controlled-release profiles (*18*, *19*). Release of carbofuran in water from CR formulations obtained through its monolithic dispersion/incorporation in carboxymethylcellulose, rosin-yellow, rosin-black, ethyl cellulose, polyvinyl acetate, cellulose acetate, polyvinyl alcohol, polyvinyl chloride, polystyrene, thermokol, bentonite, kaolinite, and Fuller's earth matrices was reported in ref *20*. The release of carbofuran from the persistent polymers was quite slow. The environmental hazards associated with a toxic pesticide such as carbofuran can be greatly reduced by formulating it as a CR formulation. Such an approach is particularly relevant in combating soilborne pests such as nematodes.

Carbofuran is the most effective insecti-nematicide used for the control of nematodes. This is relatively short-lived, and its conventional solid and liquid formulations are effective for short duration only. Its alginate-based CR formulations reported earlier are costly and not evaluated for pest control. A satisfactory control of nematodes is still a much sought after necessity of the day. In view of the prevailing menace of nematodes all over the world, the present study aimed to develop cheaper CR formulations based on sodium carboxymethylcellulose and rosin-yellow and rosin-black (obtained from the coniferous trees) for efficient nematode control.

MATERIALS AND METHODS

Test Carriers. Commercial grade bentonite (specific surface area = 575 m^2/g; differential thermal analysis: small endothermic at 160 and 700 °C, S-shaped at 850−950 °C) kaolinite (specific surface area = 34.1 m^2/g; differential thermal analysis: small endothermic at 575 °C, small exothermic at 1000 °C), and Fuller's earth (specific surface area = 510 m^2/g; differential thermal analysis: small endothermic below 150 °C, medium endothermic below 600 °C, small exothermic around 900 °C) obtained from MCA Industries (New Delhi, India) and sodium carboxymethylcellulose (viscosity = 500−800 cPs; pH 6.0−8.0 for 1% solution at 25 °C) obtained from Merck India Ltd. (Mumbai, India) were used. Rosin, a semitransparent or translucent product, is the resinous constituent of the oleoresin exuded by the conifer tree, pine (*Pinus roxburghii*, family Pinaceae). It varies in color from yellow to brown or black. Yellow and black are the commercial grades of rosin with the former being of better quality. Yellow rosin is a byproduct that remains behind after distillation of the volatile turpentine oil from pine tree resin. Black rosin is similarly obtained at high-temperature distillation and is of inferior quality. Abietic and pimeric acids are its major constituents. Rosin-black and rosin-yellow used herein were obtained from G. D. Resin (Una, Himachal Pradesh, India).

Carbofuran: molecular formula, $C_{12}H_{25}NO_3$; relative molecular mass, 221.3; melting point, 150−152 °C; vapor pressure, 0.031 mPa (20 °C); water solubility, 320 mg L^{-1} (20 °C); specific gravity, 1.18 (20 °C); K_{ow} log $P = 1.52$ (20 °C). Its technical material 85% w/w and commercial granules, 3% ai, were obtained from the manufacturer M/S Rallis India Ltd. (Bangalore, India).

Solvents and Chemicals. For routine laboratory work, laboratory grade, and for HPLC analysis, analytical grade, chemicals and solvents were employed.

Preparation of Test Products. *Rosin-Yellow- and Rosin-Black-Based Formulations.* Rosin-yellow or rosin-black (9.645 g) and carbofuran (0.355 g, 85% purity) were dissolved in acetone (40.0 mL) to obtain 3% ai concentration. The monolithically dispersed solution was thoroughly mixed and the slurry allowed to dry in a Petri dish to yield a hard mass, which was ground employing a laboratory Wiley mill and sieved to yield particles of 30/60 mesh size. The products were free flowing and yellowish or blackish in color.

Sodium Carboxynethylcellulose−Clay (Bentonite/Kaolinite/Fuller's Earth)-Based Formulations. In the preparation of polymer−clay-based formulations, the gelling properties of polymers in the presence of multivalent cations were explored. A mixture (104.25 g) of sodium carboxymethylcellulose, clay, and carbofuran technical (85% purity) was prepared in the ratio of 50:50:4.25 by weight to obtain 3% ai products and mixed in a mixer grinder. Water (250 mL) was added to make dough. To overcome stickiness, 51.0 mL of aluminum sulfate (0.5 M) was added as a gelling agent. The resultant mass (120.32 g) was dried at 40 °C in an oven. The dried mass was ground in a laboratory Wiley mill and sieved to obtain particles of 30/60 mesh size. All of the products were free flowing. The three products obtained from sodium carboxymethylcellulose along with different clays—bentonite, kaolinite, and Fuller's earth—are referred to as CMC−bentonite, CMC−kaolinite, and CMC−Fuller's earth, respectively.

Sodium Carboxymethylcellulose-Based Formulations. A mixture of sodium carboxymethylcellulose and carbofuran technical (85% purity) was prepared in the ratio of 100:4.25 by weight to obtain 3% ai products; it was mixed well in a mixer grinder and the mixture processed as for CMC−clays.

Standardization of the Carbofuran Content of the Test Formulations. Fifty milligrams of each formulation was taken in a conical flask and acetonitrile (15.0 mL) added to it. The contents were stirred in an ultrasonic bath for 10 min to achieve complete disintegration/dissolution of soluble material. After 2 h at room temperature, the mixture was sonicated twice for 10 min each and filtered quantitatively through a syringe filter (0.2 μm), and the volume was made up to 15.0 mL. Carbofuran was estimated by HPLC. The recovery of carbofuran (percent) from dry formulation was calculated as follows:

$$\text{recovery of carbofuran (\%)} = \frac{\text{amount of carbofuran in dry formulation}}{\text{amount of carbofuran in formulation processed}} \times 100$$

Recovery of carbofuran from different CR formulations varied from 94.7 to 99.8%. Rosin-yellow, rosin-black, CMC, CMC−bentonite, CMC−kaolinite, and CMC−Fuller's earth recorded values of 99.56, 99.76, 94.66, 98.40, 96.10, and 97.16% of the initially added active ingredient content. The actual carbofuran content in dry formulations of rosin-yellow, rosin-black, CMC, CMC−bentonite, CMC−kaolinite, and CMC−Fuller's earth was, respectively, 2.993, 2.989, 2.840, 2.915, 2.880, and 2.952%.

Release in Soil. In a separate study, CR formulations of carbofuran were prepared by employing different polymers/carriers, namely, carboxymethylcellulose, rosin-yellow, rosin-black, ethyl cellulose, polyvinyl acetate, cellulose acetate, polyvinyl alcohol, polyvinyl chloride, polystyrene, thermokol, bentonite, kaolinite, and Fuller's earth, and the release kinetics studied in water. The release in water was diffusion controlled with half-release times ($t_{1/2}$) in different matrices of 7.5−62.3 days. The release of carbofuran was faster from the biodegradable than persistent matrices (*20*). Only those formulations that showed adequate release of pesticide in water were selected for the soil release study.

The release of carbofuran in soil was determined as per refs *5* and *9* with minor modification. For comparison, the term ai release has been considered as the amount of active ingredient recorded at a given time. The release was compared with that of the commercial granular formulation. Typic Haplustepts soil obtained from the Institute's farm (pH 8.3; EC 0.50 ds m^{-1}; organic carbon 0.52%) was used. A known weight (∼200 mg) of CRF/granules was taken in capsules made of 5 × 5 cm parchment strips. These were placed at 1 cm depth in 25 g of soil taken in beakers (50.0 mL). Water (5.0 mL) was added to obtain 60% of water-holding capacity of the soil, and it was maintained throughout the study. The beakers were covered with Parafilm in which four small holes were made to ensure air exchange, weighed, and incubated at 30 ± 1 °C in a BOD incubator. The beakers were weighed twice a week, and water was added, as required, to compensate evaporation loss. Three beakers (three replicates) per treatment were taken periodically (1, 3, 7, 14, 21, 28, 35, and 42 days) at each sampling. The parchment capsules were removed from these beakers. Five milliliters per beaker of 10% ammonia solution was added to soil and extracted with acetonitrile/water (80:20), 3 × 15 mL. The extracts were combined, and the total volume of filtrate was noted. The filtrate was analyzed by HPLC for the carbofuran content. The soil was also fortified with a known quantity of unformulated carbofuran and extracted similarly. The recovery of carbofuran was found to be 89.2%.

HPLC Analysis. A Shimadzu high-performance liquid chromatograph (HPLC) fitted with an SPDM6A photodiode array detector was used. Samples were resolved isocratically on a 15 cm × 3.9 mm i.d. RP 18 column using acetonitrile/water (70:30) at 1 mL min^{-1} as mobile phase. The absorbance was recorded at 276 nm at a sensitivity of 0.05 AUFS by injecting a volume of 20 μL.

Analysis of the Release Data. *Diffusion Exponents.* The diffusion exponents from release data were calculated with the semiempirical power law equation as suggested in ref *21*

$$M_t/M_0 = Kt^n \tag{i}$$

where M_t/M_0 is the fraction of active ingredient released at time t, K is a constant that incorporates characteristics (porosity, tortuosity) of the macromolecular network system and the active ingredients, and n is a diffusional parameter indicative of the transport mechanism. The model was fitted by taking the logarithm on both sides of eq i

$$\log_e M_t/M_0 = \log_e K + n \log_e t + e \tag{ii}$$

The values of K and n were determined from carbofuran release data.

Time ($t_{1/2}$) for Release of 50% of Initial Carbofuran. In most cases, the rate of removal of the pesticide follows first-order kinetics. The rate of removal at a given time is directly proportional to the time. The first-order rate law is given by the equation

$$\mathrm{d}M/\mathrm{d}t = -K_r M_t \tag{iii}$$

where K_r is a rate constant, $\mathrm{d}M/\mathrm{d}t$ is the rate of removal, and M_t is the amount of pesticide present at any time t.

The integrated solution to eq iii is

$$\ln(M_t/M_\infty) = -K_t t \tag{iv}$$

M_∞ is the amount incorporated in the matrix. The rate of removal of a pesticide from the environment is often expressed as the agent's half-life $t_{1/2}$. The half-life is related to the first-order rate constant for removal, K_r, as

$$\ln 2 = -K_r t_{1/2} \tag{v}$$

or

$$K_r = (\ln 2/t_{1/2}) = (0.693/t_{1/2}) \tag{vi}$$

Prediction of Optimum Availability. The optimum availability of carbofuran from CR formulations was predicted by fitting the release data in the quadratic equation

$$\mathrm{AV_B} = a + bt + ct^2 + e \tag{vii}$$

where $\mathrm{AV_B}$ is available carbofuran, a is a coefficient of initial concentration ($t = 0$), b is the rate of change of concentration (t), c is the product of the rate of change and the rate of change of concentration ($t \times t$), and e is a constant. The maximum feasible concentration is obtained from the expression $-b/2c$. To fit into the equation, c has to be negative.

The data were analyzed using SAS PROC NLIN and PROC REG.

Bioefficacy. *Culturing of Meloidogyne incognita.* The nematode (*M. incognita*), isolated originally from a single egg mass from castor roots, was cultured, multiplied, and maintained on tomato plants raised in 30 cm diameter earthen pots containing sterilized soil−sand mixture. The egg masses were handpicked and kept on two layers of tissue paper supported by aluminum wire gauge (8−10 μm) in 10 cm dia Petri dishes filled with fresh water and kept in an incubator at 24−26 °C. Several sets of such assemblies were kept. After 4 days, the population of *M. incognita* that emerged in the suspension was determined by counting J_2 in three 1 mL aliquots three times and averaging.

Calculation of EC_{50}. Different concentrations of carbofuran (500, 400, 300, 200, 100, 50, 20, and 10 ppm) were prepared by serial dilution of the stock solution (acetone) in 0.5% emulsified water. Suspension of J_2 was diluted with water to 100 mL to obtain ≈75 juveniles mL^{-1}. To 2 mL of this nematode suspension in a Petri dish were added separately equal volumes of test solutions to obtain the desired test concentrations of 250, 200, 150, 100, 50, 25, 10, and 5 ppm, respectively. Juveniles kept in emulsified water served as control. The counts were taken at 24 and 48 h. After the required exposure, the suspension in three Petri dishes for each treatment was observed under a stereoscopic binocular microscope for determining motile and immotile counts. Juveniles found immotile were considered to be dead. The corrected percent mortality was calculated by using Abbot's formula

$$\text{corrected mortality (\%)} = [(T - \mathrm{C})/(100 - C)] \times 100$$

where T is the percent mortality in treatment and C is the percent mortality in control. $\mathrm{EC_{50}}$ values (parts per million) were calculated by using a Basic $\mathrm{LD_{50}}$ program (*22*).

Evaluation in Pots. Tomato (*Lycopersicon esculentum* L.), variety H-88, was planted in pots of 6.5 cm diameter and 9.5 cm depth. Each pot was filled with 350 g of sterilized soil. After establishment of plants in pots, the feeder roots of the seedlings were exposed by carefully removing the top layer of the soil just before inoculation. The number of juveniles per milliliter was counted before inoculation. The required quantity of nematode suspension (at 500−600 nematode larvae per pots; syn 2 J_2 g^{-1} of soil) was poured uniformly over the exposed roots and covered immediately with the topsoil. This was followed by light watering of the pots. After 24 h, each CR formulation and the commercial granular formulation at 8, 4, and 2 g per pot in three replicates were applied. An inoculated check was simultaneously maintained in which only water without the chemical was applied.

M. incognita Count in Plants and Soil. Each of the inoculated seedlings obtained from penetration (7 days) and development experiments (35 days) was removed from the pot, and the roots were gently washed in running tap water to remove adhering soil particles and stained according to the acid-fuchsin method following the technique of ref *23*. Initially, the clear root was kept in a 4% NaOCl solution for 4 min and then washed thoroughly in water to remove even the traces of chemical. Roots were then allowed to remain soaked in tap water for 3−4 h followed by dipping for 1−2 min in boiling 0.35% acid-fuschin stain. After cooling, the material was taken from the stain solution, rinsed in running tap water, and finally placed in acidified glycerol. Root pieces were pressed between glass slides for examination.

The soil from experimental pots was soaked in water for ≈4 min in a bowl. The suspension was mixed well and sieved through a series of different mesh sieves, that is, 20, 60, 100, and 400, according to Cobb's modified sieving and decanting method (*24*). Residue from the 400 mesh sieve was kept over a Petri dish assembly. The suspension was collected in a beaker and the volume made up to 100 mL. The nematode suspension was bubbled with the help of a pipet, and an aliquot of 1 mL was drawn and transferred to a counting dish to count nematodes under a stereoscopic binocular microscope. An average of five counts was taken for determining the density of larvae in suspension.

Prediction of the Duration of Action t_e. M_e is the minimum effective level ($\mathrm{EC_{50}}$) calculated separately for each controlled release formulation by using a Basic $\mathrm{LD_{50}}$ program (*22*), and M_∞ is the amount of the agent initially incorporated in the formulations. The value of M_e is the approximate value because it is based on three dosages only. The time t_e, during which an effective level of carbofuran is present after a single application, is given by

$$\ln(M_\infty/M_e) = K_r t_e \tag{viii}$$

It follows that to increase the effective duration of action t_e of conventionally applied carbofuran, its exponentially greater quantities must be applied. On the other hand, if carbofuran could be maintained at the minimum effective level, M_e, by a continuous supply to restore the fraction dissipated, then the optimum performance of a pesticide

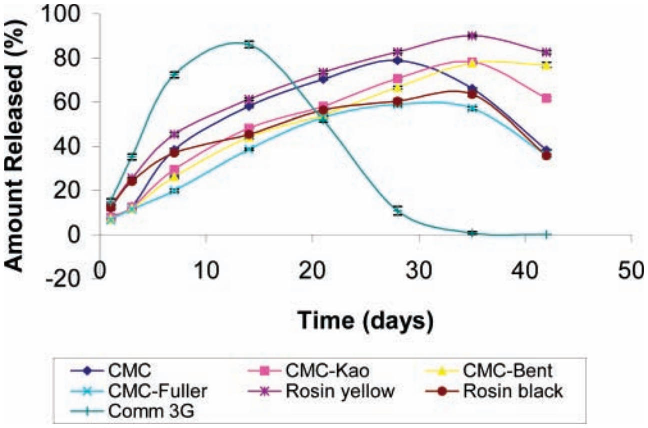

Figure 1. Cumulative release of carbofuran from controlled-release and commercial formulations in soil. Error bars represent the standard deviation of three replicates.

would be realized when the instantaneous rate of removal equals the instantaneous rate of delivery:

$$(\mathrm{d}M/\mathrm{d}t) = -K_r M_e = K_d M_e = 0 \qquad \text{(ix)}$$

K_d is the rate constant for pesticide delivery.

In CR formulations, the duration of action, t_e, of the formulation was calculated by

$$(M_\infty - M_e)/M_\infty = K_d t_e \qquad \text{(x)}$$

RESULTS AND DISCUSSION

Release Kinetics of Carbofuran in Soil. The rate of release of carbofuran in soil from the polymer/polymer−clay CR as well as the commercial 3G formulations revealed the following order:

commercial granule 3G > rosin-yellow > CMC >
CMC−kaolinite > CMC−bentonite > rosin-black >
CMC−Fuller's earth

The commercial 3G showed its maximum release on the 14th day, after which the ai content started decreasing (**Figure 1**). It became nondetectable after the 35th day. In contrast, the released amount increased gradually in the cases of all polymeric formulations up to 35 days except CMC and CMC−Fuller's earth. The rosin-yellow, rosin-black, CMC−kaolinite, and CMC−bentonite formulations showed maximum release on the 35th day, whereas CMC and CMC−Fuller's earth showed maximum release on the 28th day. In the case of CMC−Fuller's earth, the concentration of carbofuran was less than that of CMC on the 28th day. On the 42nd day the maximum concentration of carbofuran showed the following order:

rosin-yellow > CMC−bentonite > CMC− kaolinite >
CMC > CMC−Fuller's earth = rosin-black

In all of the CR products, the concentration of the ai decreased slowly after the day of its maximum release. In comparison, the decrease was at a faster pace in commercial 3G. The polymer/polymer−clay combination effectively protected carbofuran from release/degradation in soil. As long as carbofuran is entrapped in the polymeric structure, it is protected against attack by microbes as well as the other environmental degradation agents such as sunlight, water, and oxygen. The rosin-yellow polymeric formulation performed the best.

The values of K and n obtained from carbofuran released in soil are presented in **Table 1**. The mechanism of release of carbofuran in soil was similar to its release in water. The n value ranged from 0.462 to 0.740 for CR and the commercial formulations. The lowest value was obtained with rosin-black and the highest with CMC. Values close to 0.50 are stated to indicate that the release is diffusion controlled (*21*). The difference in values may be attributed to the different chemical natures of the polymers as reported for the release of diuron (*25*, *26*), imidacloprid from a lignin matrix system (*4*), and carbofuran from a modified bentonite−aliginate system (*5*).

Table 1. Constants Derived from Fitting of the Empirical Equation $M_t/M_0 = Kt^n$ to Release Data of Carbofuran in Soil from Controlled Release and Commercial Formulations

formulation	ln(*K*)	SE	*n*	SE	R^2	SE	$t_{1/2}$ (days)
CMC	2.601	0.019	0.740	0.011	0.966	0.005	12.33
CMC–kaolinite	2.643	0.031	0.689	0.011	0.981	0.001	16.08
CMC–bentonite	2.727	0.028	0.695	0.014	0.991	0.002	17.07
CMC–Fuller's earth	2.830	0.048	0.693	0.017	0.987	0.005	21.32
rosin-yellow	2.000	0.035	0.555	0.012	0.989	0.003	11.14
rosin-black	2.010	0.034	0.462	0.012	0.981	0.004	25.11
commercial 3G	1.836	0.056	0.691	0.028	0.972	0.003	4.79

Table 2. Prediction of Period of Optimum Availability (POA) of Carbofuran in Soil from Controlled-Release Formulations by Fitting the Release Data in the Quadratic Equation $AV_B = a + bx + cx^2 + e$

formulation	intercept	R^2	prob > *F*	POA (days)
CMC	0.010	0.984	0.043	25.20
CMC–kaolinite	0.011	0.981	0.040	29.20
CMC–bentonite	0.036	0.996	0.015	43.97
CMC–Fuller's earth	0.101	0.930	0.069	27.30
commercial 3G	0.083	0.947	0.042	15.10
rosin-yellow	0.120	0.988	0.011	34.30
rosin-black	0.101	0.930	0.003	26.27

The release of carbofuran in soil followed first-order kinetics. The half=release time ($t_{1/2}$) in commercial 3G formulations was only 4.79 days (**Table 1**). In CR formulations it ranged from 11.14 to 25.11 days.

In increasing order of $t_{1/2}$ the CR formulations ranked as follows:

rosin-yellow < CMC < CMC−kaolinite <
CMC−bentonite < CMC−Fuller's earth < rosin-black

The values suggested that the conventional slow-release granular product was no match for the newly developed polymeric CR products. The rate of release of toxicant from CMC has been reduced by the addition of mineral clays. Addition of montmorillonite and kaolinite clays to alginate polymer reduced the rate of release of alachlor (*3*, *27*). In the case of sodium carboxymethylcellulose also, the mechanism for reduced rate of release may be due to both an interaction of carbofuran with bentonite and kaolinite clay and an increased tortuosity resulting from disruption of the pore network in the presence of the clay.

Period of Optimum Availability (POA) of Carbofuran. The POA of carbofuran in soil from test formulations obtained by fitting the release data into a quadratic equation (POA $= a + bx + cx^2 + e$) is reported in **Table 2**. It ranged between 15.10 and 43.97 days.The minimum POA was found in the case

Table 3. EC_{50} (Parts per Million) of Controlled-Release Formulations of Carbofuran

	7th day						35th day						
	soil			plant			soil			plant			
formulation	EC_{50}	fiducial limits		EC_{50}	fiducial limits		EC_{50}	fiducial limits		EC_{50}	fiducial limits		mean EC_{50}
CMC	287.8	245.0	338.2	105.5	36.1	308.5	55.9	25.2	123.8	118.3	83.8	166.7	141.9
CMC–kaolin	367.0	327.1	411.8	266.1	208.9	336.3	66.2	33.6	130.3	154.1	115.8	245.2	213.3
CMC–bentonite	329.9	274.1	397.1	300.0	237.1	379.5	30.9	7.1	133.5	145.9	97.1	219.1	201.7
CMC–Fuller's earth	697.3	544.4	893.3	544.3	355.1	834.4	158.2	126.0	198.7	417.3	335.3	519.5	454.3
rosin-yellow	263.6	216.6	320.8	129.1	84.3	197.9	25.9	5.5	121.7	127.4	95.8	169.5	136.5
rosin-black	471.3	385.8	575.7	544.3	386.5	766.6	59.8	25.2	139.0	208.6	156.8	277.6	321.0
commercial 3G	194.7	145.7	260.3	210.7	169.0	262.6	1347.1	749.9	2419.0	730.3	523.2	1019.3	1241.4

of commercial 3G and the maximum for CMC−bentonite. The trend of POA values was as below:

commercial 3G < CMC < rosin-black <
CMC−Fuller's earth < CMC−kaolinite < rosin-yellow <
CMC−bentonite

Nematicidal Activity. *Technical Carbofuran.* The EC_{50} of technical carbofuran against *M. incognita* was found to be 125 ppm.

Controlled Release and Commercial Formulations of Carbofuran. The approximate EC_{50} values of different test formulations against *M. incognita* in soil 7 and 35 days after treatment (DAT) are reported in **Table 3**. On the 7th DAT, the commercial 3G formulation showed the lowest EC_{50} due to faster release of the ai in soil. Among the CR formulations, CMC showed the lowest and CMC−Fuller's earth the highest EC_{50} values. In the order of increasing EC_{50} value, the different test formulations ranked as follows:

commercial 3G < CMC < rosin-yellow <
CMC−bentonite < CMC−kaolinite < rosin-black <
CMC−Fuller's earth

All of the treatments provided a superior control of *M. incognita* over control at the 7th DAT.

At the 35th DAT, the EC_{50} values of different test formulations showed the following order:

rosin-yellow < CMC−bentonite < CMC < rosin-black <
CMC−kaolinite < CMC−Fuller's earth ≪ commercial 3G

The commercial 3G was inferior compared to CR formulations at 35 DAT. Degradation of carbofuran due to its faster release may be responsible for it.

Penetration and Multiplication of M. incognita. Data on the effect of formulations on the tomato root penetration and multiplication of *M. incognita* are presented in **Table 4**. All of the treatments significantly checked the nematode penetration over control at 7 DAT. The best order of checking penetration was observed as follows in the different formulations.

rosin-yellow at 8 g > commercial 3G at 8 g >
commercial 3G at 4 g > rosin-yellow at 4 g >
CMC at 8 g > CMC−kaolinite at 8 g >
commercial 3G at 2 g > CMC−bentonite at 8 g >
CMC−kaolinite at 4 g > rosin-yellow at 2 g >
CMC at 4 g > CMC−bentonite at 4 g > CMC at 2 g >
CMC−Fuller's earth at 8 g > rosin-black at 8 g >
CMC−Fuller's earth at 4 g > rosin-black at 4 g >
CMC−bentonite at 2 g > CMC−kaolinite at 2 g >
CMC−Fuller's earth at 2 g > rosin-black at 2 g > control

Table 4. Effect of Different Test Formulations on the Penetration and Multiplication of *M. incognita*

	nematode population in plant	
formulation	7 DAT	35 DAT
CMC at 8 g	62.33 (7.88)[a]	22.67 (4.74)[a]
CMC at 4 g	72.33 (8.49)	30.00 (5.45)
CMC at 2 g	97.00 (9.84)	152.67 (12.35)
CMC–kaolinite at 8 g	64.00 (7.99)	42.00 (6.47)
CMC–kaolinite at 4 g	73.67 (8.57)	55.67 (7.45)
CMC–kaolinite at 2 g	144.00 (11.99)	186.0 (13.63)
CMC–bentonite at 8 g	70.67 (8.40)	62.33 (7.89)
CMC–bentonite at 4 g	84.67 (9.19)	85.33 (9.23)
CMC–bentonite at 2 g	139.00 (11.78)	174.00 (13.18)
CMC–Fuller's earth at 8 g	100.00 (9.98)	146.33 (12.09)
CMC–Fuller's earth at 4 g	125.67 (11.21)	165.67 (12.86)
CMC–Fuller's earth at 2 g	149.67 (12.23)	276.67 (16.63)
rosin-yellow at 8 g	26.67 (5.15)	16.67 (4.07)
rosin-yellow at 4 g	51.67 (7.18)	28.67 (5.33)
rosin-yellow at 2 g	94.00 (9.69)	156.33 (12.53)
rosin-black at 8 g	100.33 (10.10)	86.00 (9.27)
rosin-black at 4 g	129.67 (11.37)	107.67 (10.37)
rosin-black at 2 g	161.67 (12.70)	210.00 (14.50)
commercial 3G at 8 g	33.33 (5.72)	298.00 (17.27)
commercial 3G at 4 g	46.67 (6.70)	318.00 (16.63)
commercial 3G at 2 g	67.33 (8.13)	349.33 (17.27)
control	219.00 (14.79)	362.33 (19.03)
CD 5%	0.859	0.822

[a] Values in parentheses are square root transformation.

The rosin-yellow at 8 g and commercial 3G at 8 g formulations were found to be most effective followed by commercial 3G at 4 g, rosin-yellow at 4 g, CMC at 8 g and, CMC−kaolinite at 8 g, with respective penetrations of 5.15, 5.72, 6.70, 7.18, 7.88, and 7.99 compared to a penetration of 14.79 in the control.

The test CR formulations also significantly controlled the nematode's repenetration and multiplication after completion of its life cycle in the following order at 35 DAT:

rosin-yellow at 8 g > CMC at 8 g > rosin-yellow at 4 g >
CMC at 4 g > CMC−kaolinite at 8 g >
CMC−kaolinite at 4 g > CMC−bentonite at 8 g >
CMC−bentonite at 4 g = rosin-black at 8 g >
rosin-black at 4 g > CMC−Fuller's earth at 8 g >
CMC at 2 g > rosin-yellow at 2 g >
CMC−Fuller's earth at 4 g > CMC−bentonite at 2 g >
CMC−kaolinite at 2 g > rosin-black at 2 g >
CMC−Fuller's earth at 2 g > commercial 3G at 8 g >
commercial 3G at 4 g > commercial 3G at 2 g > control

In the case of commercial 3G root knot nematode penetration increased from 7 to 35 DAT, whereas during the same period it decreased in most of the CR formulations. At 35 DAT, the least penetration was observed under the influence of rosin-yellow at 8 g (4.07) followed by CMC at 8 g (4.74), rosin-

Table 5. Prediction of Effective Duration (t_e) of Carbofuran from Controlled-Release and Commercial Formulations in Soil by Fitting the Mean Bioefficacy Data (EC_{50}) in the Empirical Equation $(M_\infty - M_e)/M_\infty = K_d t_e$

formulation	M_∞	M_e (EC_{50})	$(M_\infty - M_e)/M_\infty$	K_d	t_e (days)
CMC	685.7	141.9	0.79	0.06	13.2
CMC–kaolinite	685.7	213.3	0.69	0.04	17.2
CMC–bentonite	685.7	201.7	0.71	0.04	17.8
CMC–Fuller's earth	685.7	454.3	0.34	0.03	11.2
rosin-yellow	685.7	136.5	0.80	0.06	13.3
rosin-black	685.7	321.0	0.53	0.03	17.7
commercial 3G	685.7	620.7	0.09	0.14	0.7

yellow at 4 g (5.33), and CMC at 4 g (5.45) as compared to the control (19.03). The penetration and invasion by *M. incognita* juveniles were dependent on the concentration of carbofuran in the soil/plant. Under stress conditions, the multiplication and penetration of nematode were less in CR formulations as compared to commercial formulations and control.

Effective Duration (t_e) of Carbofuran from Controlled Release and Commercial Formulations in Soil. The effective duration (t_e) of carbofuran from controlled release and commercial formulations in soil is reported in **Table 5**. Fitting the mean EC_{50} values of the test formulations in the model $(M_\infty - M_e)/M_\infty = K_d t_e$ revealed that the commercial 3G was effective only for 0.7 day. Among the CR formulations, the highest effective duration of carbofuran activity was predicted for CMC−bentonite (17.8 days) and rosin-black (17.7 days) and the lowest for CMC−Fuller's earth (11.2 days).

The effective duration of carbofuran activity of different formulations was as follows:

CMC−bentonite > rosin-black > CMC− kaolinite > rosin-yellow > CMC > CMC−Fuller's earth

The increased residual toxicity of carbofuran can be exploited with advantages for nematode control. The results suggest that depending upon the matrix of polymer used, the application rate of carbofuran can be optimized to achieve nematode control at the desired level and period. A single application of the formulation can be manipulated for nematode control during the whole growth span of the crop.

NOTE ADDED AFTER ASAP PUBLICATION

The original posting of June 1, 2006, included an old version of the title. The title has been corrected with the posting of June 6, 2006.

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