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Granular Formulations of *Steinernema carpocapsae* (strain All) (Nematoda: Rhabditida) with Improved Shelf Life

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Abstract: Shelf life (nematode survival) of *Steinernema carpocapsae* (strain All) nematodes at 21 C in "Pesta" granules, made by a pasta-like process, was increased from 8 to 26 weeks by incorporating low concentrations of formaldehyde. Pesta samples containing an average of 427,000 nematodes/g were prepared with wheat flour (semolina or bread flour), kaolin, bentonite, peat moss, nematode slurry, and formaldehyde (0–1.4% w/w) and were dried to a water content of 23.6–26.9%. Nematodes emerged from Pesta (*S. carpocapsae*) granules when placed in water or on moist filter paper. Incorporation of 0.2% w/w formaldehyde (nominal; 0.05% by analysis) was optimum for increasing nematode survival in semolina-based Pesta, and also inhibited fungal growth on the granules. Bread flour Pesta samples prepared by formaldehyde addition to the nematode slurry prior to dough preparation, rather than by addition to a mixture of dry ingredients, had longer shelf life. Nematodes recovered from granules made with 0.2% formaldehyde and stored 20 weeks at 21 C caused 100% mortality of wax moth (*Galleria mellonella*) larvae.

Key words: biocontrol, entomopathogenic nematode, formaldehyde, formulation, nematode, Pesta, *Steinernema carpocapsae*, shelf life, storage, water activity, wheat flour

"Pesta" products are based on dried wheat flour dough containing living biological control agents such as fungal weed pathogens (mycoherbicides) (2,5) and entomopathogenic nematodes. Granular Pesta formulations containing *Steinernema carpocapsae* (strain All) entomopathogenic nematodes are easy to prepare and have controlled western corn rootworms (*Diabrotica virgifera virgifera*) and Colorado potato beetles (*Leptinotarsa decemlineata*) in the greenhouse (6,11). However, the wheat flour component and the high moisture content needed for prolonged nematode viability can lead to unwanted fungal and bacterial growth and spoilage of unrefrigerated Pesta formulations (6).

Formaldehyde is a broad spectrum fungicide and bactericide in use for over 100 years as a preservative in numerous products (14). Dilute formaldehyde solutions have been used for storage of nematode suspensions (3,10,12) and nematode creams (15), and in trap fluids (9). At higher concentrations, formaldehyde can function as a nematicide (8,13).

The principal objective of this study was to determine if incorporation of formaldehyde as a model antimicrobial agent would extend shelf life (nematode survival) of Pesta (*S. carpocapsae*) granules and, if so, to optimize its concentration. Another objective was to determine if high-protein bread flour could substitute for semolina as the wheat flour ingredient.

Materials and Methods

The nematode used in this study was *Steinernema carpocapsae* strain #25 (All) from biosys (Palo Alto, CA). Aqueous slurries containing between 573,000 and 620,000 live nematodes per ml (19–21% w/w) were aerated at 4 C with an aquarium pump until used in sample preparation (within 2 weeks of receipt). The number of live nematodes was counted immediately before formulation.

Preparation of Pesta granules: Semolina, a coarse (95% of particles 0.180–0.425 mm), enriched, durum wheat flour, was obtained from Tropical Nut and Fruit (Charlotte, NC), and the high-protein bread flour (enriched and bromated) used was Pillsbury brand (Minneapolis, MN) (98% of particles smaller than 0.150 mm) made from hard spring wheat. Kaolin, RC-32 type, was supplied by Thiele Kaolin (Wren, GA), and bentonite, HPM-20 type,

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was provided by American Colloid (Arlington Heights, IL). Sphagnum peat moss (Premier Brands, New Rochelle, NY) was ground and sieved to pass an 80-mesh screen. The solid ingredients in the formulation consisted of 32 g flour, 4 g kaolin, 2 g bentonite, and 2 g peat moss.

The dry solids were mixed and 35 ml of cold (10–15 C) nematode slurry was added and kneaded by hand to form a cohesive dough. Formaldehyde, as formalin solution (37.9% assay), was added to either the dry mixture of solid ingredients (dry mix) or to the aqueous nematode slurry just before dough preparation. Nominal levels of formaldehyde were 0.1, 0.2, 0.35, 0.7, and 1.4% by weight of product, assuming a 51-g sample weight after drying and no loss due to reaction or volatilization. This addition corresponded to 0.13, 0.27, 0.47, 0.94, and 1.88 g of formalin, respectively. Formaldehyde-free samples were also prepared (Table 1). Formaldehyde is a suspected human carcinogen, and proper safety measures should be employed in its handling and use.

The dough (pH 5.2–5.4) was pressed flat, folded by hand, and passed through a small pasta maker (Atlas Model 150, imported by Vitantonio Co., Eastlake, OH) set at the widest roller setting. This process was repeated until the dough appeared to be homogeneous. The dough sheet was extruded a final time at a 2-mm roller setting.

Dough sheets were covered with two paper towels and dried on a stainless steel wire mesh rack at 21 C at 65% relative humidity. The rack assembly was draped with cotton fabric to reduce airborne contamination and direct air drafts. Dough sheets were dried 16 hours, broken into several pieces, and dried uncovered until visible dampness in the center of each piece had just disappeared.

After drying, the dough was ground in a Thomas-Wiley mill, intermediate model, equipped with a 10-mesh delivery tube (Thomas Scientific, Swedesboro, NJ). The grinds were sieved to pass 10-mesh (2 mm) and collected on 18 mesh (1 mm) screens. Samples were stored in air in sealed glass vials at 21 C and in snap-cap polypropylene vials at 4 C for a total of 28 weeks. An aliquot of each sample was removed at 4-week intervals for determination of nematode viability over time (shelf life). It is likely that nematode respiration produced anaerobic conditions within the closed containers between samplings.

pH, water content, water activity, and formaldehyde determinations: The dough pH was measured using a flat-tipped combination electrode (Fisher Scientific, Pittsburgh, PA). Water content of the Pesta granules

TABLE 1. Composition of Pesta (*Steinernema carpocapsae*) granules.

Formulation†			Dried product‡		
	Formaldehyde				
Flour type	Percent§ (w/w)	Formalin (g)	H_2O (%)	Water activity (a_w)	No. live nematodes/g‖
Bread flour	0	0	24.4	0.95	414,000
	0.35	0.13	26.1	0.96	403,000
Semolina	0	0	23.6	0.95	429,000
	0.10	0.13	25.5	0.96	422,000
	0.20	0.27	25.7	0.96	438,000
	0.35	0.46	25.7	0.96	421,000
	0.70	0.92	25.9	0.95	443,000
	1.40	1.84	26.9	0.96	447,000

† The formulation consisted of 32 g flour, 4 g kaolin, 2 g bentonite, 2 g peat moss (passed 80-mesh screen) and 35 ml of nematode slurry (average of 618,000 live nematodes/ml).
‡ Average of data from 4 to 10 sample preparations.
§ The nominal amount calculated assuming a dried dough weight of 51 g and no loss due to volatilization or reaction.
‖ A calculated value assuming no loss of nematode viability during processing.

was determined by Karl Fischer titration (AquaStar V1B titrator and Model EV-6 Solid Evaporator, EM Science, Cherry Hill, NJ) using 0.18-g samples at a chamber temperature of 175 C. Water activity (a_w) of granules (1.5-g samples) was measured with a CX-1 water-activity system (Decagon Devices, Pullman, WA). Multiplication of a_w by 100 gives the relative humidity of the atmosphere in equilibrium with the sample. Water activity indicates how much free water is available to microorganisms, as opposed to water that is strongly bound to formulation components. Pesta samples were analyzed for formaldehyde by Galbraith Laboratories (Knoxville, TN) by procedure #S-540 whereby formaldehyde that leached from a 2.5-g sample immersed in water overnight was determined colorimetrically at 570 nm using chromotropic acid reagent. Also, samples were made with nematodes, but without formaldehyde, to serve as blanks for the analytical method. All analyses were run in duplicate or triplicate, and results were averaged.

Emergence and counting of nematodes: For freshly prepared samples, and subsequently at 4-week intervals, a 0.200 ± 0.002 g aliquot of each sample was weighed (8-cm square disposable weigh boat), and 10 ml of freshly drawn tap water was added. After 22–24 hours at 20–22 C, the dish was swirled to suspend the sample particles and air was pumped into the suspension using a pipet to aerate and mix the nematodes. The water-softened granules had disintegrated substantially. The suspension was transferred quantitatively to a 10-ml graduated cylinder and the volume was brought back to 10 ml (about 4–6 ml had evaporated during the soak period). The stoppered cylinder was shaken, and an aliquot was quickly removed from the middle with a pipet, diluted 10-fold if necessary, and placed in a 1-ml eelworm counting slide (Hawksley & Sons, Lancing, West Sussex, England). Live (active or "hockey-stick"-shaped) nematodes were counted and expressed as the number per gram of sample. Two aliquots from each sample were counted and averaged. In a separate experiment, a sustained release of nematodes from granules immersed in water was observed over a 3-day period (data not shown).

Assay for infectivity: Nematode infectivity was tested on wax moth (*Galleria mellonella*) larvae. A nematode suspension of 100–120 nematodes in 2 ml water was applied to 10 larvae on two layers of filter paper in a petri dish, and the larval mortality was determined after 72 hours. This method regularly provides 90–100% mortality (I. Popiel and P. Pruitt, biosys, pers. comm).

Statistical analysis: Four experimental factors were considered across time: two factors identifying formulation composition (listed in Table 1), one factor denoting the method of formulation preparation (i.e., addition of formaldehyde to either the nematode slurry or the dry mix), and a storage temperature (4 C or 21 C) factor.

The data were subjected to statistical analyses in two stages. An analysis of covariance, using time as the covariate, was conducted initially to compare the two methods of formulation preparation at each storage temperature for bread flour and for semolina. Subsequently, in the semolina formulations only, data collected using the slurry and dry-mix addition methods were combined to compare formaldehyde concentrations at 4 C and at 21 C via analysis of covariance. Before each analysis, the appropriate trend across time, the covariate, was identified by choosing the best fitting polynomial (i.e., linear, quadratic, or cubic) regression model fitted to the square root of the nematode count. Each treatment was replicated in duplicate or quadruplicate. Analyses were conducted separately for each storage temperature, because temperature had a significant effect on nematode survival (6).

The curves representing the number of live nematodes released across time were not forced through the actual number released at time zero. Hence, the method of least-squares regression was used to fit the data. To determine treatments yielding the better (longer) shelf life, regression es-

timates of model parameter (e.g., mean number of emerged live nematodes and rate of decrease in number of live nematodes across time) were compared for equality among treatments. The r^2 value of the regression model fitted to each treatment is reported to indicate the proportion of the total data variability explained by the model and, hence, $(1 - r^2)$ is attributable to error.

Results

Pesta (*S. carpocapsae*) samples contained an average of 427,000 nematodes/g (Table 1). Incorporation of 2 g bentonite in the dough formulation (about 3.9% of the final product) in place of the same amount of kaolin (Table 1 and ref. 6) increased by 40% the amount of nematode slurry that could be used and still make a cohesive dough. However, dough cohesiveness decreased at higher bentonite levels, making it difficult to obtain an intact dough sheet. Throughout the 28-week test period, there was no loss of water activity for samples stored at 4 C and only about a 0.01 a_w loss at 21 C.

Formaldehyde addition to bread flour formulations: The order of addition of formalin solution to bread flour Pesta formulations had a significant impact on subsequent nematode survival over time (shelf life). When 0.35% formaldehyde was added to the dry mix (r^2 = 0.68), i.e., bread flour, kaolin, bentonite, and peat moss, shelf life of the nematodes in the Pesta granules at 21 C was shorter (P = 0.0082) than when formalin was added directly to the aqueous nematode slurry (r^2 = 0.95) (Fig. 1). For example, 0 vs. 70,000 nematodes/g, respectively, survived 16-week storage. Regardless of order of addition, nematode survival was superior for formaldehyde-containing samples compared to formaldehyde-free (r^2 = 0.75) samples ($P <$ 0.0322).

Refrigeration extended shelf life substantially, as anticipated from earlier work (6). Shelf life differences for bread flour samples containing 0.35% formaldehyde

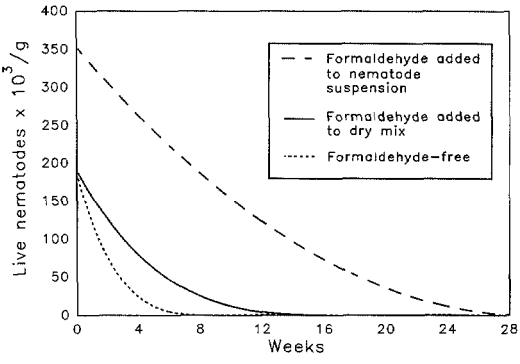

FIG. 1. Effect of the order of addition of 0.35% (w/w; nominal) formaldehyde on shelf life (number of live nematodes that emerged per gram of sample vs. time) at 21 C in the preparation of bread flour Pesta (*S. carpocapsae*) granules.

and made by each of the addition methods and stored at 4 C were marginal (P = 0.0677). As observed at 21 C, formaldehyde extended shelf life, and formalin addition to the nematode slurry immediately prior to dough preparation was more effective (r^2 = 0.39) than addition to the dry mix (r^2 = 0.30). Higher live nematode counts were obtained with formaldehyde-containing samples than with formaldehyde-free (r^2 = 0.63) samples (Fig. 2) (P = 0.0760 dry mix, P = 0.0214 wet slurry).

Formaldehyde addition to semolina formulations: Semolina-containing Pesta formulations were not as sensitive as bread flour formulations to formaldehyde addition order. The data plotted in Fig. 3 indicate a shorter shelf life at 21 C for nematodes in

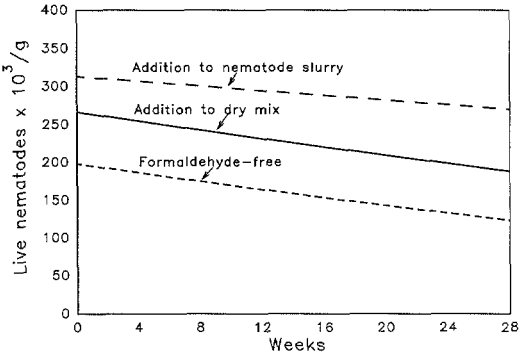

FIG. 2. Effect of the order of addition of 0.35% (w/w; nominal) formaldehyde on shelf life at 4 C in the preparation of bread flour Pesta (*S. carpocapsae*) granules.

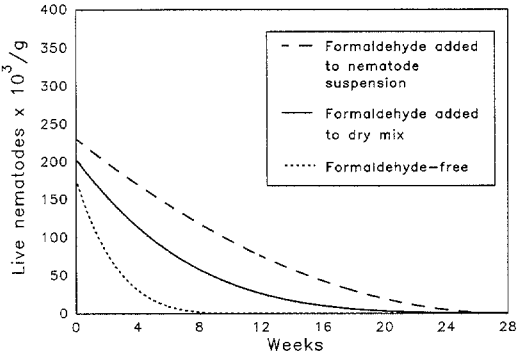

FIG. 3. Effect of the order of addition of 0.35% (w/w; nominal) formaldehyde on shelf life at 21 C in the preparation of semolina Pesta (*S. carpocapsae*) granules.

samples made by formalin addition to the semolina-containing dry mix (r^2 = 0.70) compared with addition (0.35% formaldehyde) to aqueous nematode slurry (r^2 = 0.87), but the difference was not significant (P = 0.1596). However, formaldehyde incorporation by either addition order prolonged shelf life significantly compared with formaldehyde-free (r^2 = 0.77) samples ($P < 0.0243$).

At 4 C, results with semolina Pesta were nearly identical, regardless of formaldehyde addition order (r^2 = 0.43, dry; r^2 = 0.48, slurry) and refrigeration prolonged shelf life substantially (Fig. 4). Nematode survival over 28 weeks was similar for samples with or without (r^2 = 0.76) formaldehyde.

Wheat flour type was not a significant factor affecting nematode survival over time at 21 C for formaldehyde-free samples. When 0.35% formaldehyde was incorporated by addition to nematode slurry, bread flour samples (r^2 = 0.91) gave no better nematode survival than semolina (r^2 = 0.87) throughout the 28-week test. However, semolina (r^2 = 0.70) was marginally better (P = 0.0840) than bread flour (r^2 = 0.68) when formaldehyde was added to the dry mix. With 4 C storage, bread flour (r^2 = 0.63) was no better than semolina (r^2 = 0.76) for samples lacking formaldehyde, but was better (P = 0.0214) for samples where formalin was added to the nematode slurry (r^2 = 0.39, bread flour; r^2 = 0.48, semolina). Flour type was not a significant factor for samples made by formaldehyde addition to the dry mix and stored at 4 C. About 50,000 live nematodes/g were extracted from samples made with 0.35% formaldehyde and stored 1 year at 4 C.

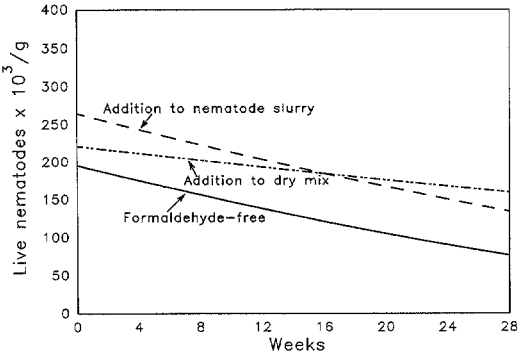

FIG. 4. Effect of the order of addition of 0.35% (w/w; nominal) formaldehyde on shelf life at 4 C in the preparation of semolina Pesta (*S. carpocapsae*) granules.

Effect of formaldehyde concentration on semolina formulations: Formaldehyde was incorporated in semolina Pesta (*S. carpocapsae*) granules at 0, 0.1, 0.2, 0.35, 0.7, and 1.4% (w/w; nominal), and the samples were stored at 21 C and 4 C. Data from both methods of formaldehyde addition (to dry mix and to nematode slurry) were combined. At 21 C, there was a gradual and total loss of nematode viability by 28 weeks for all the samples, but between 0 and 28 weeks there were significant differences in viability attributable to formaldehyde content (Fig. 5). The 0.2% level (r^2 = 0.78) maintained more live nematodes (about 90,000/g at 12 weeks) than any of the other concentrations ($P < 0.0476$). The 0.35% (r^2 = 0.63) and 0.7% (r^2 = 0.61) levels, the next most effective concentrations, gave equivalent results (about 30,000/g at 12 weeks). The 0.1% (r^2 = 0.78) and 1.4% levels (r^2 = 0.57), and the formaldehyde-free sample were least effective at maintaining viable nematodes (0 to 10,000/g at 12 weeks) during 21 C storage. Fungal growth was not observed on samples containing at least 0.2% formaldehyde.

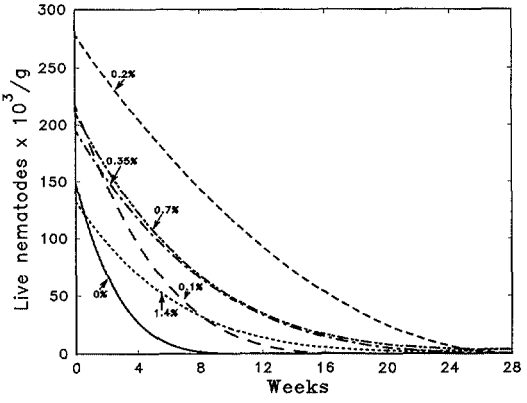

FIG. 5. Effect of formaldehyde concentration incorporated in semolina Pesta (*S. carpocapsae*) on shelf life at 21 C.

Nematode viability during 4 C storage was also affected by formaldehyde concentration in the Pesta granules (Fig. 6). At 0.1% and 0.2%, initial levels of viable nematodes were maintained over the 28-week test. The 0.35% samples (r^2 = 0.26) were the next most effective. The 0.7% (r^2 = 0.31) samples did not differ from the 0% (r^2 = 0.38) formaldehyde samples. Loss of viable nematodes was most pronounced for the 1.4% (r^2 = 0.55) formaldehyde samples.

Formaldehyde analysis of Pesta granules: Only 20–29% of the formaldehyde incorporated into the semolina Pesta (*S. carpocapsae*) granules was detected by analyses conducted 1 to 3 weeks after preparation and refrigerated storage (Table 2). The

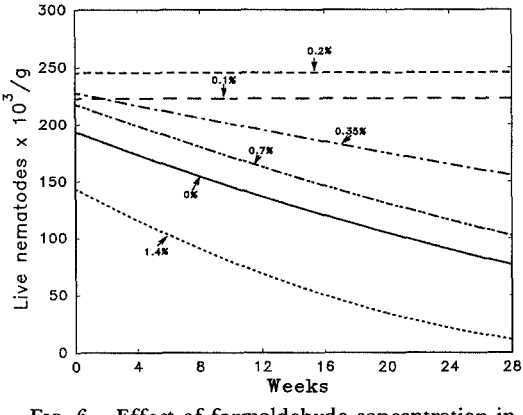

FIG. 6. Effect of formaldehyde concentration incorporated in semolina Pesta (*S. carpocapsae*) on shelf life at 4 C.

TABLE 2. Formaldehyde analyses of semolina Pesta (*Steinernema carpocapsae*) granules.

Formaldehyde, % (w/w)†	
Added	Found‡
0.10	0.02
0.20	0.05
0.35	0.08
0.70	0.17
1.40	0.41

† Overnight aqueous leach, 2.5 g in 50 ml water, chromotropic acid reagent, colorimetric analysis at 570 nm.

‡ Subtracted blank value of 0.08%; average of 2 or 3 determinations.

lowest percentage of added formaldehyde was recovered from the 0.1% samples and the highest from the 1.4% samples. Samples prepared with nematodes, but without formaldehyde, analyzed 0.08% formaldehyde, which served as the blank value for the analytical method.

Infectivity of nematodes released from formaldehyde-containing Pesta: Nematodes released from Pesta granules containing 0.1% to 1.4% formaldehyde and stored 8 weeks at 21 C caused 100% mortality of wax moth larvae (with typical infection by the bacterial associate). Nematodes from samples containing 0.2% formaldehyde stored 20 weeks at 21 C also caused 100% larval mortality. No larvae were killed by exposure for 4 days to 0.1 g of nematode-free Pesta containing 0, 0.35, and 1.4% formaldehyde.

DISCUSSION

As an alternative to using a more concentrated nematode slurry, the number of nematodes per gram of Pesta can be increased by incorporating water-holding ingredients in the formulation. Dough made with bentonite (ca. 3.9% of the final product) effectively accommodated more aqueous slurry than dough made with 100% kaolin as the clay component. High final water content and water activity were known to prolong shelf life of Pesta granules (6). Therefore, samples were prepared with as high a water activity (Table 1) as practical for dough processing and

grinding. These high moisture levels were easier to achieve when dough sheet thickness was increased from about 1.1 (6) to 2.0 mm, because this slowed the drying rate.

Pesta (*S. carpocapsae*) granules were prepared under non-sterile conditions and, unless an antimicrobial agent was added, fungi and bacteria frequently grew under the favorable conditions of high water activity and 21 C storage (6). Fungal growth caused clumping of the granules and loss of their free-flowing property. Incorporation of only 0.2% w/w (nominal; 0.05% actual) formaldehyde in semolina Pesta extended shelf life (measurable nematode viability) at 21 C from about 8 weeks to 26 weeks. Formaldehyde levels up to 0.35% also improved refrigerated shelf life. It is not certain from this study if the benefits observed with formaldehyde are due solely to its antimicrobial properties or if another mechanism is involved.

The greatly reduced beneficial effect on shelf life that resulted from formaldehyde addition to the dry mix of bread flour Pesta, versus addition to nematode slurry, was probably due to reaction of formaldehyde with proteins in the finely ground flour (7). The small amount of formalin solution added was rapidly absorbed in a localized portion of the flour-containing mixture where some reaction could have occurred before the aqueous nematode slurry was added to prepare the dough. Formaldehyde lost by reaction or otherwise bound to a small portion of the flour would not be available to function as a preservative. In contrast, addition of formalin to the nematode slurry uniformly exposed the nematodes to formaldehyde before contact with the flour. Semolina Pesta was affected less because this flour's coarse particle size was much greater than that of bread flour, so reaction would proceed more slowly and to a lesser extent. Even with semolina, it would be preferable to add formalin to the nematode slurry to be assured of optimum results.

Bread flour samples made by formalin (0.35% formaldehyde) addition to the nematode slurry gave no better shelf life results than the equivalent semolina samples. Hence, both flours were suitable for use in Pesta formulations, and factors such as cost or dough-forming properties under process conditions would dictate the choice. Other flours or flour mixtures may also prove to be acceptable. Exclusive of formaldehyde level, the formulations were not optimized. The ability to modify the ratios of ingredients and incorporate new additives are attractive features of Pesta formulations. Previous work (6) has shown that water activity and storage temperature affected shelf life much more than the composition of the formulations tested.

The nominal 0.2% formaldehyde level in semolina Pesta (*S. carpocapsae*) granules that gave the best shelf life at 4 C and 21 C actually contained only 0.05% formaldehyde by weight. Samples with a nominal 0.35% level, which also had good shelf life, analyzed 0.08% formaldehyde (Table 2). Higher formaldehyde levels either gave no greater benefit or were harmful. Minimizing formaldehyde content in products is prudent because it is a potential human carcinogen as well as an irritant and sensitizer. Differences between the amount of formaldehyde added to the samples and the amount found by analysis probably are due to losses through volatility and reaction with flour proteins.

Wax moth larval mortality results showed that formaldehyde did not harm the symbiotic *Xenorhabdus nematophilus* bacteria harbored by the infective-stage *S. carpocapsae* juveniles. Bacteria can survive even chlorination inside the bodies of certain nematodes (4).

In Pesta granules, nematode movement is restricted, conserving energy reserves of the non-feeding, infective juveniles. The clay components can adsorb potentially toxic excretory products and, together with the flour, release water slowly during drying to allow the nematodes to physiologically prepare to enter a resting stage (1).

Pesta (*S. carpocapsae*) granules are free-flowing and easy to apply and incorporate

in soil. Granular formulations of entomopathogenic nematodes are not available commercially, and would be an attractive alternative or supplement to products designed for spray application to soil. Extending shelf life at 21 C, as reported in this work, is one step toward the goal of a commercially attractive granular product for insect biocontrol with nematodes.

Literature Cited

1. Bedding, R. A. 1988. Storage of entomopathogenic nematodes. International Patent WO 88/08668.

2. Boyette, C. D., H. K. Abbas, and W. J. Connick, Jr. 1993. Evaluation of *Fusarium oxysporum* as a potential bioherbicide for sicklepod (*Cassia obtusifolia*), coffee senna (*C. occidentalis*), and hemp sesbania (*Sesbania exaltata*). Weed Science 41:678–681.

3. Capinera, J. L., D. Pelissier, G. S. Menout, and N. D. Epsky. 1988. Control of black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae), with entomogenous nematodes (Nematoda: Steinernema, Heterorhabditidae). Journal of Invertebrate Pathology 52:427–435.

4. Chang, S. L., G. Berg, N. A. Clarke, and P. W. Kabler. 1960. Survival and protection against chlorination, of human enteric pathogens in free-living nematodes isolated from water supplies. American Journal of Tropical Medicine and Hygiene 9:136–142.

5. Connick, W. J., Jr., C. D. Boyette, and J. R. McAlpine. 1991. Formulation of mycoherbicides using a pasta-like process. Biological Control 1:281–287.

6. Connick, W. J., Jr., W. R. Nickle, and B. T. Vinyard. 1993. "Pesta": New granular formulations for *Steinernema carpocapsae*. Journal of Nematology 25: 198–203.

7. Fraenkel-Conrat, H., and H. S. Olcott. 1948. The reaction of formaldehyde with proteins. V. Crosslinking between amino and primary amide or guanidyl groups. Journal of the American Chemical Society 70:2673–2684.

8. Giblin-Davis, R. M., J. L. Cisar, and F. G. Bilz. 1988. Evaluation of three nematicides for the control of phytoparasitic nematodes in 'Tifgreen II' bermudagrass. Supplement to the Journal of Nematology 2:46–49.

9. Howell, J. F. 1979. New storage methods and improved trapping techniques for the parasitic nematode *Neoaplectana carpocapsae*. Journal of Invertebrate Pathology 33:155–158.

10. Kung, S-p, R. Gaugler, and H. K. Kaya. 1990. Influence of soil pH and oxygen on persistence of *Steinernema* spp. Journal of Nematology 22:440–445.

11. Nickle, W. R., W. J., Connick, Jr., and W. W. Cantelo. 1994. Effects of Pesta-pelletized *Steinernema carpocapsae* (All) on western corn rootworms and Colorado potato beetles. Journal of Nematology, 26: 249–250.

12. Poinar, G. O., Jr. 1975. Entomogenous nematodes. Leiden: E. J. Brill.

13. Stapleton, J. J., B. Lear, and J. E. DeVay. 1987. Effect of combining solarization with certain nematicides on target and nontarget organisms and plant growth. Supplement to the Journal of Nematology 1:107–112.

14. Walker, J. F. 1975. Formaldehyde, 3rd. ed. Huntington, NY: R. E. Krieger.

15. Yukawa, T. 1985. Nematode storage and transport. International Patent WO 85/03412.