Efficacy of biologically active agents as nontraditional nematicides for *Meloidogyne javanica*

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SUMMARY

Some 20 physiologically active agents including antibiotic and anthelminthic drugs, plant growth regulators and inhibitors, medicinal and natural plant extracts and antimetabolites have been tested as nematode control agents. The inhibitors were applied as soil drenches or as root-dips. As drenches, six inhibitors reduced a nematode population to less than 15 % of controls; as root-dips five inhibitors reduced nematode population levels to less than 15 % of controls; only one inhibitor was common to both categories. Given the current climate, nematode control with available chemicals will be an increasing difficulty. There is great need to discover new modes of action different from the one now targeted with nonfumigant nematicides. This approach expanded more thoroughly in the future may in part help resolve the nematode control question.

RÉSUMÉ

Efficacité d'agents biologiquement actifs comme nématicides inhabituels contre Meloidogyne javanica.

Quelque vingt agents biologiquement actifs comprenant des antibiotiques et des antihelminthiques, des régulateurs ou inhibiteurs de la croissance végétale, des extraits végétaux médicinaux et naturels et des antimétabolites ont été testés pour leur action contre les nématodes. Ces produits ont été appliqués soit en tranchées dans le sol, soit en trempage des racines. Dans le premier cas, six des produits ont réduit la population du nématode à moins de 15 % du témoin; en trempage de racines, cinq produits réduisent la population de nématodes à moins de 15 % du témoin; un seul des produits était commun aux deux catégories. La conjoncture rendra de plus en plus difficile les traitements à l'aide des nématicides chimiques actuels. Il est donc nécessaire de découvrir des modes d'action différant de ceux recherchés actuellement pour les nématicides non fumigants. Si une telle approche se développait plus complètement dans le futur, elle pourrait permettre de résoudre, en partie, la question de la lutte contre les nématodes.

Traditional chemical control using the nematicides available for the last few decades is in a declining status internationally. Health and hazard concerns which have elicited close scrutiny by regulatory agencies have resulted in increased restrictions or prohibitions of use. There is a growing need to develop new nematicides, less hazardous to health, despite the negative economic reality of doing so that discourages commercial activity. The second generation nematicides (nonfumigant nematicides) target one physiological reaction, the inhibition of acetylcholine esterase activity, one step of a series that involves nerve impulse conduction. In view of the general acceptance of the involvement of evolutionary processes in adaptation to selective pressure, it is not surprising that a wide range of organisms including arthropods, microorganisms, weeds, and nematodes have been found to modify their properties in response to long-term pesticide stress. This population adaptation often takes the form of tolerance or indifference to the pesticide and frequently to other pesticides whose mode of action is the same (NRC, 1986).

It is generally accepted that chemical methods of pest control will be needed for the foreseeable future. To meet this need, pest control technology must deal with the dynamic process that renders good pesticides useless. Nematologists, therefore, must seek ways to reduce the exclusive dependence upon agents with one mode of action and to search for others with diverse modes of action to add to the available options. It has been said (Campbell, 1983) that the most productive search for new drugs for infectious diseases has utilized the empirical approach, e.g., all successful classes of anthelminthic, antibacterial and antiprotozoal drugs have been discovered by empirical testing. The second school of thought advances the rational approach which in essence is predicated upon the discovery, in a parasite, of a biochemical pathway or event that might be blocked without harm to the host. Elements of both approaches have been used in the search for plant-parasitic nematode controls; fumigant nematicides were found with the empirical approach; nonfumigant nematicides were adopted from findings in insect control which in turn

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Table 1

Biologically active agents and alleged modes of action

Inhibitor	*Activity*
1) Ampicillin. 6- [(Aminophenylacetyl) amino] -3, 3-dimethyl-7-oxo-4-azabicyclo [3.2.0] heptane-2-carboxylic acid	Antibacterial inhibition of R-factor determinants in certain bacteria
2) L-Thiazoline-4-carboxylic acid	Inhibits L-proline uptake by *Pseudomonas aeruginosa*
3) 3-Isobutyl-1-methylxanthine	Inhibits cyclic nucleotide phosphodiesterase, tumor colony formation
4) Methylglyoxal bis- (guarylhydrazone dihydichloride) monohydrate	Inhibits S-adenosyl-methionine decarboxylase; polyamine metabolism
5) 5-Thio-D-glucose	Competitively inhibits cellular D-glucose transport; inhibits spermatogenesis; with hypothermia kills hypoxic tumor cells selectively
6) Fertilysin™; N, N'-1,8-Octane diylbis [2,2-dichloroacetamide]	Inhibits spermatogenesis without affecting gonadotropin production
7) 3-Methyl-2-cyclohexene-1-one	Anti-aggregative hormone
8) Terephthalic acid	Inhibits spontaneous mice mammary tumorigenesis
9) Quercetin dihydrate	Inhibits lactate transport, glycolysis in asite tumor cells, phosphodiesterase
10) Phenyldichlorophosphate	Inhibits acetylcholinesterase
11) Kinetin riboside	Anticancer, antiviral agent
12) Tilerone analog R; 3,6-bis [2- (dimethylamino) ethyoxy] -9H-xanthen-9-one dihydrochloride	Interferon induction; antiviral, anticancer agent
13) *all trans* - Retinoic acid	Involved in cellular differentiation in whole animal; inhibits tumor cell proliferation; depending on concentration, enhances or inhibits induction of gonadotropin receptors; inhibits phorbolmyristate acetate stimulated superoxide production from nutrophils; may inhibit collagen production
14) Tetramisole hydrochloride DL-2, 3, 5, 6-tetrahydro-6-phenylimidazo [2, 1-b] thiazole	Broad spectrum anthelmintic; inhibits alkaline phosphatase, fumarate reductase
15) Ftorafur. 5-fluro-1- (tetrahydro-2-furfuryl) uracil	Tumor growth suppressor
16) Abamectin. Mixture of Avermectin B,'s-macrocylic lactones from *Streptomyces avermitilis*	Anthelmintic activity; stimulates release of γ-aminobutyric acid, an inhibitory neurotransmitter in arthropods
17) Citral. 3,7-Dimethyl-2,6-octadienal	Possible precursor in vitamin A synthesis; essential oil in perfumery
18) Geraniol. 3,7-Dimethyl-2,6-octadien-1-ol	Insect attractant; possible precursor to higher active terpenoids
19) Neem seed extract *(Azadirachta indica)*	Natural extract with insecticidal activity
20) Triton-100	Surfactant

were based upon the empirical approach. In the special case of nematodes where only one biochemical event has been targeted for blockage, it may be useful to take advantage of blocking agents for different events, discovered with other organisms, for semi-empirical testing.

This intermediate approach involving elements of the purely empirical and rational ones has been used by nematologists for the last two decades. For example, to inhibit the formation of giant cells in galls, essential for the development and reproduction of *Meloidogyne* spp., by application of plant growth regulators and retardants (Davide & Triantaphyllou, 1968; Mukhopadhyaya & Krishnamoorthy, 1971; Orion & Minz, 1971; Orion, 1974; Mjuge & Viglierchio, 1976; Ganguly & Dasgupta, 1984*a*, *b*), of metabolic inhibition (Bird & McGuire, 1966; Burton & Schaeffer, 1967; Estey & Panayi, 1972; Arrigoni *et al.*, 1979; Sawhney & Webster, 1979; Sitaramaiah & Pathak, 1979; Evans, 1984; Glazer & Orion, 1985), of antibiotics (Estey & Panayi, 1972), of anthelminthics (Birtle *et al.*, 1982; Sasser *et al.*, 1982; Gara-

Table 2

Trial I : *Meloidogyne javanica* egg production on tomato after application of inhibitors as soil drenches at different times relative to inoculation (Egg masses/plant-EM/P; eggs/egg mass-E/EM. Duncan's multiple range test; values with same letter do not differ at 95 % level)

		Preinoculation		*Inoculation*		*Postinoculation*		
Inhibitor	*Conc. PPM*	*EM/P*	*E/EM*	*EM/P*	*E/EM*	*EM/P*	*E/EM*	*Rel. Reprod. potential*
1	400	493 *b*	159 *a*	244 *d*	203 *b*	160 *f*	155 *a*	25
2	400	301 *e*	143 *ab*	244 *d*	132 *c*	205 *e*	149 *ab*	31
3	400	409 *c*	140 *ab*	248 *cd*	126 *c*	400 *b*	138 *abc*	56
4	400	409 *c*	121 *bc*	46 *g*	122 *c*	295 *d*	137 *abc*	41
5	400	182 *g*	107 *cd*	130 *e*	103 *d*	152 *f*	136 *bc*	21
6	400	464 *b*	95 *de*	297 *b*	99 *d*	400 *b*	120 *cd*	49
7	400	478 *b*	89 *def*	277 *bc*	97 *d*	331 *c*	120 *cd*	40
8	400	417 *c*	87 *def*	87 *f*	93 *d*	118 *g*	113 *de*	14
9	400	355 *d*	74 *efg*	249 *cd*	91 *d*	293 *d*	105 *de*	31
10	400	234 *f*	64 *fg*	254 *cd*	85 *de*	351 *c*	99 *ef*	35
11	400	3 *h*	58 *g*	13 *h*	70 *e*	10 *h*	87 *f*	0.9
Control	—	772 *a*	166 *a*	398 *a*	270 *a*	637 *a*	155 *a*	100

bedian & Van Gundy, 1983; Israr *et al.*, 1984; Nanje & Setty, 1984; Nordmeyer *et al.*, 1985), of plant extracts (Taylor & Murant, 1966; Kali & Gupta, 1980; Mukhopadhyaya *et al.*, 1980; Singh *et al.*, 1980; Haroon & Smart, 1983*a*, *b*; Prot & Kornprobst, 1983; Hasan & Jain, 1984).

This report concerns experiments seeking to extend the past studies of untested agents and previously tested and untested agents applied in a different way by using the *Meloidogyne javanica*-tomato system. Evaluations are directed at agents able to intercede in the host symptomatic response as well as the development or reproduction of the nematode.

Materials and methods

Susceptible tomato (*Lycopersicon esculentum* Mill.) cv. U.C. 82 was germinated in a sterilized sandy soil mixture (1 : 1 river sand and sandy loam). The biologically active agents selected as test materials indicated in Table 1 were arbitrarily divided into two trials; each trial involved a drench and a root-dip treatment. For drench treatments, approximately 40-day-old seedlings were transplanted into 5 cm foam cups filled with sterilized soil mixture and allowed to establish for approximately one week. All inoculations involved newly hatched second-stage juveniles of *M. javanica* at the rate of 1 000/pot. For the root-dipping treatments, plants were removed from the germinating bed, the roots washed in a stream of water, then placed in the inhibitor baths for 20 hours before removal and transplanting as before. Nematode inoculations took place after the plants were established for several days.

In the drench treatment of Trial I, inhibitor solutions were applied either three days before inoculation, at inoculation, or three days after inoculation to assess the protective nature of the plant root to endoparasitic nematodes established in the root tissue. After two months of growth, the roots of each plant were washed in a stream of water, then examined with the aid of a microscope to determine the number of egg masses/root system; a random sample of egg masses were similarly examined to determine the number of well-developed eggs/egg mass. Inhibitor application rates are indicated in the results of Tables 2-5 (four replicates/treatment). The three portions of these treatments were slightly staggered to facilitate examination of fresh material in the bioassays. Relative reproductive potential was estimated by $\left(\text{EM/P} \times \frac{\text{E}}{\text{EM}}\right)$ of treatment relative to control, or in Table 5 EM/P of treatment relative to control.

Results

The effect of inhibitors selected for Trial I, in terms of egg masses/plant and eggs/egg mass, when applied as drenches before inoculation, at inoculation, and after inoculation, are indicated in Table 2. In all cases, egg masses/plant were suppressed significantly from controls. Whereas, eggs/egg mass were suppressed by seven inhibitors at preinoculation treatments, all inhibitors at inoculation treatments, and six inhibitors at postinoculation treatments. It is of interest to note that egg masses/plant were reduced below 50 % of controls

by five inhibitors at preinoculation treatments, four inhibitors at inoculation treatments, and seven inhibitors at postinoculation treatments. In terms of eggs/egg mass, three inhibitors reduced the number to less than 50 % of controls for preinoculation treatments, ten inhibitors did so at inoculation treatments whereas, no inhibitor in postinoculation treatment was able to reduce egg/egg mass below 50 % of controls.

The efficacy of the inhibitors employed in Trial II as drenches (Tab. 3) in terms of egg masses/plant and eggs/egg mass were significantly below controls. In 14 of 31 treatments, egg masses/plant were less than 50 % of controls; in terms of eggs/egg mass, 12 of 31 were less than 50 % of controls. As with Trial I drench treatments, certain inhibitors were much more effective than others.

Trial I inhibitors, tested as root-dips (Tab. 4), indicated a significant decrease ($P = 0.01$) in egg masses/plant in 11 of 26 treatments, whereas, in terms of eggs/egg mass, a similar reduction appeared in 9 of 26 treatments.

For insight into two additional parameters possibly impinging upon the effects of Trial II inhibitors used as root-dips, inoculations were conducted three weeks after treatment, rather than less than one as in Trial I, and efficacy was assessed as galls/plant and egg masses/plant. Unfortunately, it was not possible to assess eggs/egg mass as before. In fifteen of sixteen treatments, galls/plant or egg masses/plant were significantly below controls (Tab. 5).

Discussion

The results herein confirm an inability to predict efficacy of a biologically-active agent on the basis of simple properties, e.g., biological activity or structure. Empirical testing which encompasses a number of parameters, e.g., movement in soil, agent stability, penetration of host or parasite tissue, and target of activity among others, all critical components which must be overcome, has been able to reveal effective agents with practical potential to merit further consideration.

Although egg mass production was generally reduced in comparison to controls, several inhibitors effected a marked reduction. The egg/egg mass production, always less than controls, varied widely with treatment. With some inhibitors, e.g., tetramisole, ftorafur, and abamectin, the egg masses were very small as if these inhibitors reduced gel matrix production as well. In all treatments, galls formed randomly except for the kinetin riboside, where galls formed in clusters. In most cases, there was a positive correlation between increasing concentration and the terminal nematode level, except for certain inhibitors (citral and geraniol), in which there was no additional effect. In all treatments, shoot growth was not significantly different from controls, except for three root-dip treatments which were phytotoxic (Tab. 4).

Table 3

Trial II : *M. javanica* egg production on tomato after application of inhibitors as soil drenches one week after inoculation (Egg masses/plant-EM/P; eggs/egg mass-E/EM Duncan's Multiple Range Test; values with same letter do not differ at 95 % level)

Inhibitor	*Conc. PPM*	*EM/P*	*E/EM*	*Rel. reprod. potential*
11	12.5	127 *q*	341 *l*	12
	25	73 *r*	265 *no*	5
12	100	281 *ijk*	281 *n*	22
	200	264 *jk*	251 *op*	18
	400	231 *lm*	296 *r*	13
13	100	217 *mn*	312 *m*	19
	200	182 *op*	169 *no*	14
	400	65 *r*	200 *r*	4
14	100	256 *kl*	593 *q*	42
	200	226 *lm*	522 *fg*	33
	400	213 *mno*	405 *j*	24
15	100	283 *ijk*	702 *b*	55
	200	212 *mno*	674 *c*	39
	400	96 *qr*	550 *de*	15
16	100	—	516 *g*	
	200	230 *lm*	455 *h*	29
	400	308 *ghi*	380 *k*	32
17	100	382 *cd*	452 *h*	48
	200	365 *cde*	401 *j*	40
	400	355 *cef*	350 *l*	34
18	100	210 *mno*	244 *p*	14
	200	188 *nop*	195 *r*	10
	400	170 *p*	151 *s*	7
19	125	397 *bc*	540 *ef*	59
	250	312 *ghi*	435 *i*	37
	500	286 *ijk*	418 *j*	33
	1 000	416 *b*	402 *j*	46
	2 000	211 *mno*	407 *j*	24
20	125	336 *efg*	432 *i*	40
	250	323 *fgh*	404 *j*	36
	500	326 *fgh*	396 *j*	36
Control	—	480 *a*	754 *a*	100

The nematode levels obtained with drench tests of eleven inhibitors applied before, at, and after inoculation (Tab. 2) when transformed to a common denominator, indicated that in certain cases the plant root may have accorded some protection to the endoparasitic nematode population. Although the protection afforded by the root tissue is a factor, it appeared to be a minor one. The calculated relative reproductive potential determined at harvest, relative to controls, whether in terms of egg numbers (Tabs 2, 3, 4), or egg masses (Tab. 5) varies enormously from 1 to 190 % of controls. According to this criterion, certain treatments increased the reproductive potential substantially, while others decreased it drastically. If the old guideline for chemical control of

Table 4

Trial I : inhibitors used as root dips to affect *M. javanica* reproduction on tomatoes * Indicate significant difference from controls at 1 % level, indicates phytotoxicity

Inhibitor	*Conc. PPM*	*Egg masses plant*	*Eggs Egg mass*	*Rel. reprod. potential*
1	100	426	51*	31
	200	379	50*	27
	400	343*	46*	22
2	100	326*	99*	46
	200	264*	52*	20
	400	144*	31*	6
3	100	570	169	137
	200	334*	162	77
	400	292*	156	65
4	100	—	—	
5	100	286*	180	73
	200	203*	135	39
	400	—	—	
6	100	523	167	124
	200	492	191	134
	400	468	219	146
7	100	533	128	97
	200	502	222	159
	400	482	268	184
8	100	517	185	136
	200	483	161	111
	400	449	147	94
9	100	562	184	147
	200	534	176	134
	400	494	169	119
10	100	181*	31*	8
	200	132*	45*	8
	400	84*	25*	3
11	100	—	—	
Control	—	450	156	100

Table 5

Trial II : inhibitors used as root dips to affect *M. javanica* reproduction on tomatoes Duncan's multiple range test; values followed by same letter do not differ at 95 % level

Inhibitor	*Conc. PPM*	*Galls plant*	*Egg masses plant*	*Rel. reprod. potential*
11	12.5	177 *fg*	20 *hi*	15
	25	176 *fg*	8 *ij*	6
	50	157 *g*	4 *j*	3
12	100	223 *e*	106 *cd*	82
13	100	320 *a*	174 *a*	134
	200	187 *f*	102 *cd*	78
14	100	244 *cd*	17 *ij*	13
15	100	176 *fg*	78 *e*	60
	200	165 *g*	44 *fg*	34
16	100	76 *h*	32 *gh*	25
	200	31 *i*	131 *ij*	10
	400	11 *i*	4 *j*	3
17	100	322 *a*	114 *c*	88
	200	272 *b*	96 *d*	74
	400	223 *de*	71 *e*	55
18	100	264 *bc*	54 *f*	42
Control	—	316 *a*	130 *b*	100

nematodes in invoked, i.e., " a treatment effecting 85 % or better reduction in nematode population levels can be considered useful ", five inhibitors, viz. terephthalic acid, kinetin riboside, tilerone, retinoic acid, ftorafur and geraniol merit further study as drenches. As root-dips, five inhibitors, viz. thiazoline carboxylic acid, phenyl dichlorophosphate, kinetin riboside, tetramisole and abamectin merit further study. Evident as well is the fact that the inhibitor root dips of Trial II were effective on nematodes inoculated three weeks after treatment and remained so for an additional two months to harvest. Apparently, only kinetin riboside was useful as a drench and as a root-dip. It is premature to speculate on mode of action on the basis of current knowledge when, for example, one inhibitor, terephthalic acid, stimulates a reproductive potential above controls as a root-dip, but reduces it below 15 % as a drench. Moreover, another inhibitor, methylcyclohexene-1-one depresses the reproductive potential somewhat as a drench, but at a high dose as a root-dip, can double it. A similar effect is observed with isobutylmethylxanthine. Whether the inhibitor acts by interfering with the host processes in the root tissue response, thereby stressing the nematode, which in turn reduces egg potential, or whether the inhibitor interferes directly with the nematode processes, is irrelevant for practical purposes so long as the nematode population level is reduced sufficiently. Theoretically, the mode of action is of great importance, for an improved understanding of activity may reveal completely new classes of useful compounds. We consider this a useful area of research where, the observations of many science man years of research with other organisms can be utilized for the benefit of nematode control. Moreover, since some of these compounds have therapeutic use, the health hazard aspect may be somewhat diminished.

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