

Nematicidal effects of some plant-extracts to *Aphelenchoides composticola* (Nematoda) infesting mushroom, *Agaricus bisporus*

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SUMMARY

Leaf, flower or seed-extracts of 29 dominant plant species found in northern India were screened against adult and juvenile stages of *Aphelenchoides composticola* infesting white button mushroom, *Agaricus bisporus*. Leaf-extracts of *Bougainvillea spectabilis*, *Calotropis procera*, *Cedrella toona*, *Jacaranda acutifolia*, *Melia azadirach*, *Ricinus communis* and *Tagetes patula* and seed-extracts of *M. azadirach* and *R. communis* were highly toxic to this nematode. Nematicidal activity in leaf-extracts of most plant species varied with the method of extract preparation. Two statistical models, viz., analysis of variance and probit analysis, were compared to test the effectiveness of the extracts. The role of incorporating dried leaves of pre-tested plants in mushroom compost for management of *A. composticola* populations is also discussed.

RÉSUMÉ

Action nématicide de quelques extraits végétaux envers *Aphelenchoides composticola* (Nematoda) infestant le champignon de couche, *Agaricus bisporus*

Des extraits provenant de feuilles, de fleurs ou de graines de plantes dominantes dans le nord de l'Inde ont été testés envers les adultes et les stades juvéniles d'*Aphelenchoides composticola*, infestant le champignon de couche, *Agaricus bisporus*. Les extraits foliaires de *Bougainvillea spectabilis*, *Calotropis procera*, *Cedrella toona*, *Jacaranda acutifolia*, *Melia azadirach*, *Ricinus communis* et *Tagetes patula*, et les extraits provenant de semences de *M. azadirach* et de *R. communis* sont très toxiques pour le nématode. L'action nématicide des extraits foliaires de la plupart des espèces végétales varie suivant la méthode de préparation. Deux modèles statistiques (analyse de variance et analyse "probit") ont été comparés pour les tests d'activité des extraits. L'incorporation de feuilles séchées de plantes pré-testées au compost des champignonnières pour contrôler les populations d'*A. composticola* est également discutée.

Mycophagous nematodes *Aphelenchoides composticola*, *A. sacchari* and *Ditylenchus myceliophagous* are reported to cause heavy losses in yields of mushroom, *Agaricus bisporus* in commercial farms in India (Chhabra & Kaul, 1982; Sharma, Thapa & Nath, 1981). Because of the lack of bulk pasteurization facilities and the endemic and acute nature of the nematode problem in some potential and traditional mushroom growing areas (including Himachal Pradesh, Jammu and Kashmir, Punjab and Haryana), some growers are closing down their mushroom farms (Thapa, Sharma & Nath, 1981; Grewal & Sohi, 1987a). Both *A. composticola* and *A. sacchari* cause up to 100 % loss in mushroom yield depending upon the initial inoculum and time of introduction into the compost (Grewal, 1987; Sharma, Thapa & Kaur, 1985). Damage thresholds of *A. composticola* are so low that even a single nematode per 200 g of

compost at the time of spawning can lead to significant loss in yield (Arnold & Blake, 1968; Grewal, 1987).

Although nematicides including aldicarb and phorate (Cayrol & Ritter, 1972; Chhabra & Kaul, 1981), benomyl, fenamiphos, oxamyl and thiabendazole (McLeod & Khair, 1978), carbofuran, diazinon and dichlorvos (Grewal & Sohi, 1987a) have been shown to control *A. composticola* in mushrooms, recent reports have illustrated toxic effects of pesticides on mushroom mycelium and losses in yield (Grewal & Sohi, 1987b; Grewal, Upadhyay & Sohi, 1988; White, 1986). Furthermore, the use of systemic nematicides in mushroom culture results in toxic residues in the produce (Bahl & Agnihotri, 1987).

Plant-extracts nematicidal against *A. composticola* (Seth *et al.*, 1986; Grewal & Sohi, 1988) would be a cheaper and effective alternative. Grewal (1988) dem-

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onstrated that the incorporation of dried leaves of four different plants to mushroom compost resulted in the suppression of *A. composticola* populations below economic injury level and in an increase in the nitrogen content of the compost and the yield of the mushroom, *Agaricus bisporus*. The present paper reports the results of further trials wherein most of the dominant plant species (with abundant foliage) from northern India were screened to test the leaf, seed or flower-extract toxicity against *A. composticola*.

Materials and methods

PREPARATION OF EXTRACTS

Leaf-extracts of 29 plant species viz., *Allium cepa*, *Althea rosea*, *Azadirachta indica*, *Araucaria sp.*, *Bauhinia variegata*, *Bougainvillea spectabilis*, *Brassica campestris*, *Callistemon lanceolata*, *Calotropis procera*, *Cannabis sativa*, *Cedrella toona*, *Cedrus deodara*, *Chenopodium album*, *Chrysanthemum cinerariifolium*, *Clerodendron infortunatum*, *Curcuma longa*, *Dahlia variabilis*, *Ficus hispida*, *Jacaranda acutifolia*, *Lantana camara*, *Melia azadirach*, *Morus alba*, *Nerium indicum*, *Pinus roxburghii*, *Populus ciliata*, *Ricinus communis*, *Tagetes patula*, *Thuja sp.* and *Zingiber officinale* were prepared both in cold and hot water.

Cold water leaf-extracts (CWE) were prepared by blending the leaves of test plants in distilled water (100 g/500 ml) in a mixer at 25° for 2 min. The extracts were then filtered through a Whatman filter paper No. 1 and stored at 5° until needed. Hot water leaf-extracts (HWE) were prepared by boiling the leaves of test plants in distilled water (100 g/500 ml) for 20 min. After cooling, the extracts were filtered as described above, the volume adjusted to 500 ml and stored at 5°.

Hot water extracts were also prepared from the seeds of *A. indica*, *C. lanceolata*, *M. azadirach* and *R. communis* and also from the flowers of *B. spectabilis*, *C. cinerariifolium*, *D. variabilis* and *T. patula*.

NEMATODE CULTURE

Aphelenchoides composticola Franklin was cultured in glass bottles on grain spawn of the mushroom, *Agaricus bisporus* (Lange) Singer (Strain S11) as described by Grewal (1988). The nematodes were extracted from the infested spawn using Baermann funnel (Hooper, 1986). The nematode suspension with all life stages was then calibrated.

SCREENING FOR NEMATICIDAL EFFECTS

Extracts were used at 2, 5 or 10 % concentration (dilutions in distilled water). One ml of the diluted extract was added to a cavity block containing 110 (\pm 12) nematodes in one ml of distilled water. Controls

were run by adding 1 ml distilled water to the nematode suspension. There were seven treatments replicated three times in a factorial design : cold or hot water extract, three concentrations and a distilled water control. The cavity blocks were covered and incubated at 25° for 48 hours. Leaf-extracts (both CWE and HWE) of only one plant species were tested at a time. Nematicidal effects of seed or flower-extracts (only HWE) were tested similarly (three concentrations and one control).

While recording the data on nematode mortality, the active lethal effects (nematicidal) were distinguished from the nematostatic effects (suppression of nematode movement). The extract solution in cavity block was diluted (1:128) with distilled water, and the numbers of dead/live nematodes were recorded after 24 h.

ANALYSIS OF DATA

Data for each species were analysed by probit analysis and analysis of variance (after arcsin transformations). The hot and cold water assays were tested for differences using parallel probit regression analysis (Ross, 1980). Estimates of LC₅₀ (as percentages) and the slope of each assay are presented in Tables 1 & 2.

Tests of significance are based on the chi-squared statistic :

- (a) heterogeneity : tests for consistency of replicates and fit of the model;
- (b) parallelism : tests for parallelism of probit regression line;
- (c) position : tests for differences in LC₅₀ assuming a parallel response.

Results

LEAF-EXTRACTS

The leaf-extracts of seventeen plant species, out of 29 tested, showed nematicidal effects against *A. composticola*. The data on per cent nematode mortality caused by the cold or hot water formulations of the leaf-extracts of these plants are presented in Fig. 1.

Cold water preparations of the leaf-extracts of *C. procera* and *J. acutifolia* caused 100 % nematode mortality at 5 % concentration. Furthermore, 100 % mortality was also produced by cold water extracts of *B. spectabilis*, *C. toona*, *M. azadirach*, *R. communis* and *T. patula* at 10 % concentration. Hot water leaf-extracts of only *C. procera*, *J. acutifolia* and *R. communis* caused 100 % nematode kill at 10 % concentration.

Results from the probit analysis and of the analysis of variance of the data are presented in Table 1. The efficacy of the leaf-extracts and of their formulations were compared at two levels : mean overall effect at 2-10 % concentration by the analysis of variance and at the level of 50 % nematode kill (LC₅₀ values) by the probit analysis.

Table 1
Effects of leaf-extracts on *A. composticola* : probit analysis and analysis of variance

Plant species	Probit analysis							Analysis of variance		TxC int. ^x
	Cold water		Hot water		Significance			Extract		
	LC50 (%)	Slope	LC50	Slope	Position	Parallel- ism	Hetero- geneity ^s	Type	Conc.	
<i>Althea rosea</i>	19.2 ⁺	1.92	4.6	3.72	***	***	ns	***c	***	***
<i>Azadirachta indica</i>	3.1	2.36	4.2	2.69	***	ns	ns	ns	***	ns
<i>Bougainvillea spectabilis</i>	2.6	3.24	75.9 ⁺	1.22	***	***	ns	***	***	***
<i>Callistemon lanceolata</i>	11.1 ⁺	1.77	7.4	2.49	***	*	*	ns	***	ns
<i>Calotropis procera</i>	< 2.0 ⁺	nd	1.4 ⁺	2.50	*	***	ns	***	***	***
<i>Cannabis sativa</i>	9.1	2.45	3.2	3.44	***	***	*	***	***	***
<i>Cedrella toona</i>	2.8	4.64	43.4 ⁺	1.41	***	***	ns	***	***	***
<i>Chenopodium album</i>	3.6	2.41	4.0	4.56	***	***	***	ns	***	***
<i>Chrysanthemum cinerarifolium</i>	5.4	2.13	5.7	2.61	ns	ns	*	ns	***	ns
<i>Dahlia variabilis</i>	0.4	0.86	> 10.0 ⁺	nd	***	ns	*	***	**	ns
<i>Jacaranda acutifolia</i>	2.0	nd	0.7 ⁺	1.37	***	***	***	ns	***	***
<i>Lantana camara</i>	1.9 ⁺	2.00	4.4	3.18	***	***	***	***	***	***
<i>Melia azadirach</i>	0.1 ⁺	0.58	2.8	3.86	***	***	ns	***	***	***
<i>Pinus roxburgii</i>	1.0 ⁺	1.45	3.4	3.92	***	*	***	***	***	***
<i>Populus ciliata</i>	9.1	1.26	12.4 ⁺	1.95	***	*	ns	ns	***	ns
<i>Ricinus communis</i>	1.0 ⁺	2.07	0.8 ⁺	2.13	*	ns	***	ns	***	ns
<i>Tagetes patula</i>	3.5	4.41	22.4 ⁺	2.29	***	*	ns	***	***	***

ns : not significant (P > 0.05); * P < 0.05; ** P < 0.01; *** P < 0.001

nd : not determined-failure of probit analysis to converge

⁺ : LC₅₀ outside the range 2-10 %

^s : heterogeneity significance was in all cases due to lack of fit of the model and not variation between replicates

^x : Type x Concentration interaction

Table 2

Effects of seed- or flower-extracts on *A. composticola* :
Probit analysis and analysis of variance

	Probit analysis			Analysis of variance
	LC ₅₀ (%)	Slope	Hetero- geneity	
SEED EXTRACTS				
<i>Azadirachta indica</i>	3.7	3.82	***S	***
<i>Melia azadirach</i>	< 2.0 ⁺	nd	nd	***
<i>Ricinus communis</i>	< 2.0 ⁺	nd	nd	***
FLOWER EXTRACTS				
<i>Bougainvillea specta- bilis</i>	8.6	3.68	ns	***
<i>Chrysanthemum ci- nerarifolium</i>	8.7	3.62	ns	***
<i>Tagetes patula</i>	9.1	1.95	ns	***

ns : not significant (P > 0.05); *** P < 0.001

nd : not determined-failure of probit analysis to converge

⁺ : LC₅₀ outside the range 2-10 %

S : Heterogeneity significance was due to lack of fit of the model and not variation between replicates

Leaf-extracts (both cold or hot water preparations) of the twelve plant species viz., *A. cepa*, *Araucaria* sp., *B. variegata*, *B. campestris*, *C. deodara*, *C. infortunatum*, *C. longa*, *F. hispida*, *M. alba*, *N. indicum*, *Thuja* spp. and *Z. officinale* showed no or little (< 40 %) nematode mortality even at 10 % concentration.

SEED OR FLOWER-EXTRACTS

Hot water preparations of the seed-extracts of three out of the four plant species tested were highly toxic to the target nematode (Fig. 2). Seed-extracts of *M. azadirach* (at 10 % concentration) and *R. communis* (at 5 and 10 % concentration) caused 100 % nematode mortality. Seed-extracts of *A. indica* caused more than 80 % nematode kill at and above 5 % concentration. However, *C. lanceolata* showed little effect (maximum mortality, 44 %).

Flower-extracts (only hot water formulations tested) of three plant species out of the four species screened, showed nematocidal effects on the test nematode (Fig. 2). Although none of the plant species caused complete mortality at the concentrations tested (2-10 %), more than 50 % nematode kill resulted from the flower-ex-

tracts of *B. spectabilis*, *C. cinerarifolium* and *T. patula* at 10 % concentration. *D. variabilis* showed little effect on the target nematode (maximum mortality, 26 %).

Results of the probit analysis and of the analysis of variance for both seed- and flower-extracts of these plants are summarised in Table 2.

Discussion

Nemato-toxic compounds in the leaf extracts of the seventeen plant species out of 29 tested listed in Table 1 were water-soluble and could be filtered, in contrast to that of garlic (*Allium sativum*) in which the toxins are water insoluble and cannot be filtered through Whatman filter paper No. 1 (Nath *et al.*, 1982). However, differential effects of leaf extracts of the plants tested and of their formulations (cold or hot water extracts) on nematode mortality suggest that the active nematicidal principles in various plants could be different. On the basis of the effects of cold or hot water extracts on nematode mortality (when the data were processed for analysis of variance, Tab. 1), the plant species tested could be grouped into four distinct categories as follows :

- (i) Where hot and cold water extracts were equally effective, indicating the thermostable nature of their active principles, e.g. *Azadirachta indica*, *Callistemon lanceolata*, *Chenopodium album*, *Chrysanthemum cinerarifolium*, *Jacaranda acutifolia*, *Populus ciliata* and *Ricinus communis*. However, the probit analysis revealed that the LC₅₀ values for cold of hot water assays varied significantly for all plant species except *C. cinerarifolium* (Tab. 1). This showed that although the two formulations of a leaf-extract do not differ significantly, the concentrations at which 50 % of the individuals die (LC₅₀ values) may be variable.
- (ii) Where nematicidal activity was observed in cold water extract but was less (i.e. max. 25.6 % nematode mortality) in the hot water extracts showing the thermolabile nature of the nemato-toxic principles, e.g. *Bougainvillea spectabilis*, *Cedrella toona*, *Dahlia variabilis* and *Tagetes patula*. Grewal and Sohi (1988) observed that the nematicidal compounds in the leaves of *Tectona grandis* were also heat-labile.
- (iii) Where hot water extracts, possessed nematicidal activity but were significantly ($p < 0.001$) less toxic than cold water extracts, e.g. *Calotropis procera*, *Lantana camara*, *Melia azadirach* and *Pinus roxburgii*. The reason for such an effect is not known, but may be due to a minor change brought by heat in the chemical nature of active principle.
- (iv) Where hot water extracts were significantly ($p < 0.001$) more toxic than cold water extracts, e.g. *Althea rosea* and *Cannabis sativa*. Such an effect may be due either to a favourable chemical change or that the heat enhanced the extraction of active ingredients

from the leaves. Both of these possibilities have been supported by Egunjobi and Afolami (1976) who observed an increase in the toxicity of neem leaf-extracts to *Pratylenchus brachyurus* after boiling.

Present findings revealed that the seed-extracts of *A. indica*, *M. azadirach* and of *R. communis* are highly toxic to *A. composticola*. Recently, Devakumer, Goswami and Mukerjee (1985) observed that limonoids (compounds belonging to B-furano-triterpenoids) present in the kernels of neem (*A. indica*) are the main nematotoxic principles. However, no such active nematicidal compounds have yet been identified from the seeds of *M. azadirach* and *R. communis*.

The use of two different statistical models for analysis of the data helped to elucidate some of the relative advantages and disadvantages of the two models. Although LC₅₀ values are generally used to compare the

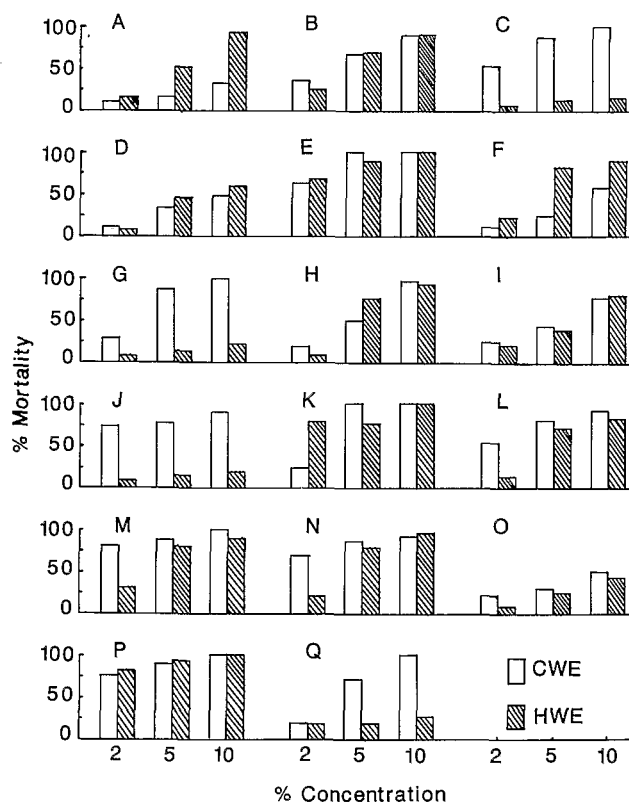


Fig. 1. Mean % mortality (corrected according to Abbot, 1925) of *A. composticola* at 2, 5 and 10 % concentration of cold water (CWE) and hot water (HWE) prepared leaf-extracts of various plant species after 48 h : A : *Althea rosea*; B : *Azadirachta indica*; C : *Bougainvillea spectabilis*; D : *Callistemon lanceolata*; E : *Calotropis procera*; F : *Cannabis sativa*; G : *Cedrella toona*; H : *Chenopodium album*; I : *Chrysanthemum cinerarifolium*; J : *Dahlia variabilis*; K : *Jacaranda acutifolia*; L : *Lantana camara*; M : *Melia azadirach*; N : *Pinus roxburgii*; O : *Populus ciliata*; P : *Ricinus communis*; Q : *Tagetes patula*.

relative toxicities of leaf-extracts and of their formulations but it does not depict or take into consideration the response of the nematode exposed to higher doses of the extract. The latter type of effect is better depicted by the analysis of variance. As is evident from the data, that in some cases e.g. cold water leaf-extracts of *D. variabilis* (Tab. 1), 50 % kill of the nematode was produced at a concentration of 0.4 % but complete mortality was not achieved even at 10 % concentration. Whereas in others, e.g. cold water leaf-extracts of *J. acutifolia*, the LC_{50} value was much higher (2 %) but complete mortality was observed at 5 % concentration. In other words, the straight line probit model does not fit in well where the quantal response is truly sigmoid. In such cases, the significance of heterogeneity resulted from the lack of fit of the model, i.e. due to the failure of the probit analysis to converge (Tab. 1 & 2). Conversely, when the quantal response is linear, the probit model is the best which also predicts the LC_{50} values even outside the range of the concentrations tested.

Apart from the nematicidal activity, the leaf extracts of some of the above plants were shown to possess other beneficial effects including increase in the mycelial growth of *A. bisporus* (Sohi, Grewal & Seth, 1987) and fungi-suppressive effects to common mushroom weed moulds (Grewal & Grewal, 1988). Furthermore, Grewal (1988) demonstrated that the incorporation of dried leaves of *A. indica*, *C. sativa*, *Eucalyptus* hybrid (*E. tereticornis*) and *R. communis* to the mushroom compost significantly reduced *A. composticola* populations, improved physico-chemical characteristics of compost and

increased yield of *A. bisporus*. Sohi, Grewal and Seth (1987) and Grewal (1988) also observed that the leaf-matter incorporation in compost increased the populations of nematode-trapping/antibiotic-producing fungi and suppressed that of competitor/pathogenic moulds. It was therefore suggested that a range of interrelated factors including leaf-extract toxicity, leaf decomposition products (supported by Sayre, Patrick and Thorpe, 1965), activities of nematode-trapping fungi and fungal-antibiosis were responsible for the suppression of *A. composticola* populations below economic injury levels.

It is therefore concluded that the incorporation of dried leaves of pre-selected plants (screened for their nematicidal effects) could provide a suitable and cheaper alternative to biologically manage *A. composticola* infesting the Indian mushroom industry. Although the active nematicidal principles from the leaf-extracts of the plant species screened in the present study are yet to be characterized, these findings will enable us to test the above theories in different geographical regions of the country using locally available leaf-matter.

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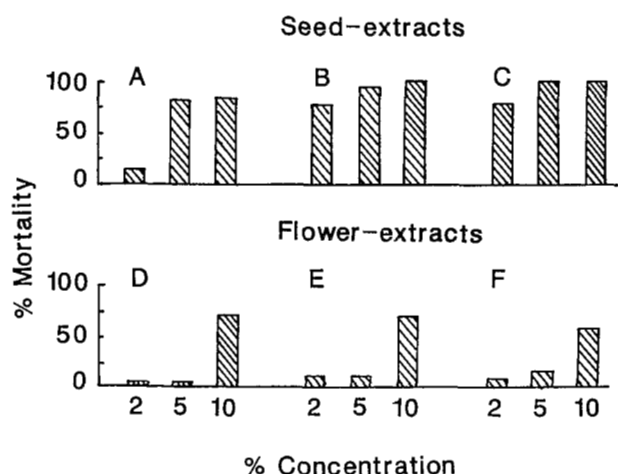


Fig. 2. Mean % mortality (corrected according to Abbot, 1925) of *A. composticola* at 2, 5 and 10 % concentrations of hot water prepared seed- or flower-extracts of various plant species after 48 h : A : *azadirachta indica*; B : *Melia azadirach*; C : *Ricinus communis*; D : *Bougainvillea spectabilis*; E : *Chrysanthemum cinerariifolium*; F : *Tagetes patula*.

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