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

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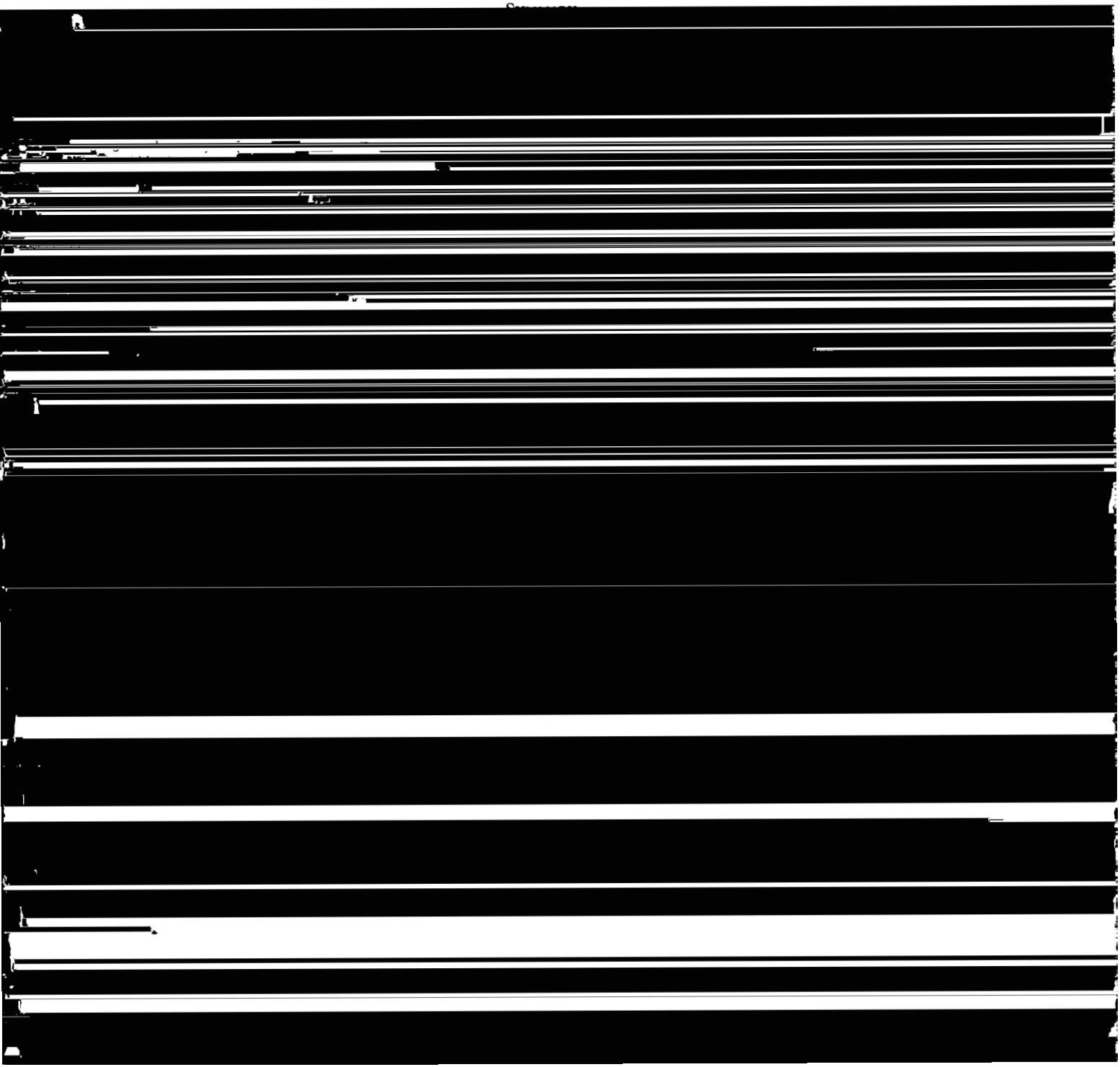

onstrated that the incorporation of dried leaves of four different plants to mushroom compost resulted in the suppression of *A. composticola* populations below economic injury level and in an increase in the nitrogen content of the compost and the yield of the mushroom, *Agaricus bisporus*. The present 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 cinerarifolium, Clerodendron infortunatum, Curcuma longa, Dahlia variabilis, Ficus hispida, Jacaranda acutifolia, Lantana camara, Melia azadirach, Morus alba, Nerium indicum, Pinus roxburgii, 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. commu-*

[redacted]

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 LC_{50} (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 LC_{50} assuming a parallel response.

Results

Leaf-extracts

The leaf-extracts of seventeen plant species, out of 29 tested, showed nematicidal effects against *A. composti-*

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

Plant species	Probit analysis							Analysis of variance		
	Cold water		Hot water		Significance			Extract		TxC int.[x]
	LC50 (%)	Slope	LC50	Slope	Position	Parallelism	Heterogeneity[S]	Type	Conc.	
Althea rosea	19.2[+]	1.92	4.6	3.72	***	***	ns	***[c]	***	***
Azadirachta indica	3.1	2.36	4.2	2.69	***	ns	ns	ns	***	ns
Bougainvillea spectabilis	2.6	3.24	75.9[+]	1.22	***	***	ns	***	***	***
Callistemon lanceolata	11.1[+]	1.77	7.4	2.49	***	*	*	ns	***	ns
Calotropis procera	< 2.0[+]	nd	1.4[+]	2.50	*	***	ns	***	***	***
Cannabis sativa	9.1	2.45	3.2	3.44	***	***	*	***	***	***
Cedrella toona	2.8	4.64	43.4[+]	1.41	***	***	ns	***	***	***
Chenopodium album	3.6	2.41	4.0	4.56	***	***	***	ns	***	***
Chrysanthemum cinerarifolium	5.4	2.13	5.7	2.61	ns	ns	*	ns	***	ns
Dahlia variabilis	0.4	0.86	> 10.0[+]	nd	***	ns	*	***	**	ns
Jacaranda acutifolia	2.0	nd	0.7[+]	1.37	***	***	***	ns	***	***
Lantana camara	1.9[+]	2.00	4.4	3.18	***	***	***	***	***	***
Melia azadirach	0.1[+]	0.58	2.8	3.86	***	***	ns	***	***	***
Pinus roxburgii	1.0[+]	1.45	3.4	3.92	***	*	***	***	***	***
Populus ciliata	9.1	1.26	12.4[+]	1.95	***	*	ns	ns	***	ns
Ricinus communis	1.0[+]	2.07	0.8[+]	2.13	*	ns	***	ns	***	ns
Tagetes patula	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 analysisto converge
[+] : LC_{50} 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	Heterogeneity	
SEED EXTRACTS				
Azadirachta indica	3.7	3.82	***[S]	***
Melia azadirach	< 2.0[+]	nd	nd	***
Ricinus communis	< 2.0[+]	nd	nd	***
FLOWER EXTRACTS				
Bougainvillea spectabilis	8.6	3.68	ns	***
Chrysanthemum cinerarifolium	8.7	3.62	ns	***
Tagetes patula	9.1	1.95	ns	***

ns : not significant (P > 0.05); *** P < 0.001
nd : not determined-failure of probit analysis to converge
[+] : LC_{50} 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 nematicidal 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

Nematotoxic 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 [illegible] 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*

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

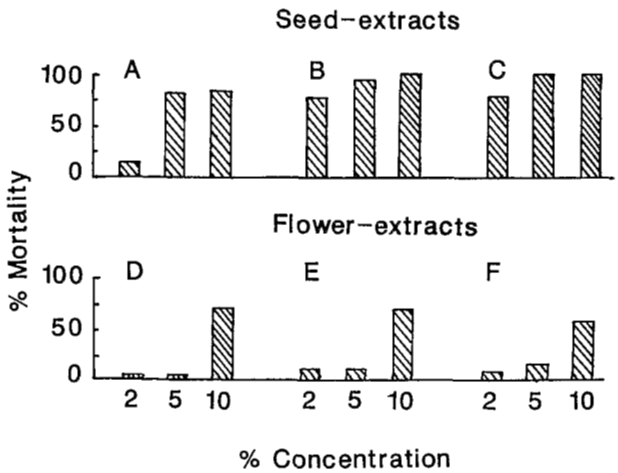

Fig. 2. Mean % mortality (corrected according to Abbot, 1925) of *A. composticola* at 2, 5 and 10 % concentrations of hot water prepared seed- or flower-extracts of various plant species after 48 h : A : *azadirachta indica;* B : *Melia azadirach;* C : *Ricinus communis;* D : *Bougainvillea spectabilis;*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

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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