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Allelopathic effects of *Eupatorium riparium* on population regulation of two species of *Galinsoga* and soil microbes

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Summary Allelopathic effect of *Eupatorium riparium* Regel, a dominant ruderal weed at higher altitudes in Meghalaya state in north-eastern India, was studied on two common sympatric annual weeds, *Galinsoga ciliata* (Raf.) and *G. parviflora* Cav. and on soil microbes. Seed germination and radicle and plumule growth of both species of *Galinsoga* were suppressed by the aqueous extract and leachate of *E. riparium*. Although the leaf leachate, leaf and litter extracts and litter bed caused considerable reduction in leaf area and seed and dry matter production in both species of *Galinsoga*, the effect was much more pronounced on *G. parviflora*. The inhibitory effect was directly correlated with the concentration of the extract and leachate. The soil microbial population and growth of the *Galinsoga* spp. declined considerably in the experimental pots where the soil had earlier received leachate of different plant parts of *E. riparium* growing in it. The presence of the partly decomposed litter of *E. riparium* in the pots reduced soil microbial population and growth of the two weeds much more strongly as compared to the litter in the advanced stages of decomposition. The study also revealed that the abundance and colony growth of the two test fungi *viz. Trichoderma viride* and *Aspergillus flavus* were differentially affected by the allelopathy of *E. riparium*; *T. viride* being favoured and *A. flavus* inhibited.

Introduction

Inhibitory effect of chemicals released by plants into the environment has been demonstrated by several workers on the neighbouring plant species[22,30]. Changes in community structure and species-dominance have also been attributed to allelopathy in several studies[4,5,9,12,14,16,19,21]. However, the role of allelopathy in regulating the populations of weedy species and soil microbes has not been emphasized much.

The dominance of *Eupatorium riparium* Regel, a noxious ruderal weed of family Asteraceae over other plant species in nature has been attributed to its allelopathic effects[18]. Certain plant species especially the two annual weeds, *Galinsoga ciliata* (Raf.) Blake and *G. parviflora* Cav. (Asteraceae), showed suppressed growth and reduced population density when grown in the neighbourhood of *E. riparium*. This leads to the assumption that *E. riparium* has some regulatory effect on the

populations of the two species of *Galinsoga*. Thus, a study was carried out to examine the allelopathic effects of *E. riparium* on germination and growth of the two species of *Galinsoga*. The investigation was also extended to study the effects of *E. riparium* on soil microbial population.

Materials and methods

Experiment 1: Effect of E. riparium *extracts and leachates on seed germination and radicle and plumule growth of* Galinsoga *spp.*

Fresh leaves and litter of *E. riparium* were collected from field populations in May 1980 and aqueous extracts of 1% and 5% concentrations were obtained by crushing 1 and 5 g of the leaves and litter in 100 ml distilled water with a pestle and mortar and by filtering the crushed material through a muslin cloth. The leaf leachate was prepared by shaking mechanically 1 and 5 g of fresh leaf with 100 ml distilled water for 2 h. The freshly collected fully ripened seeds of *G. ciliata* and *G. parviflora* were soaked in leaf extracts/leachates of different concentrations for 24 h. The corresponding controls, with the seeds of the two species soaked in distilled water for the same period, were also maintained. Fifty soaked seeds of each of the two species were kept in Petri dishes on moist filter paper for germination, and for each treatment three replicates were maintained. All the Petri dishes were kept in a BOD incubator at 25 ± 2°C. Seed germination was recorded over a 15 days period after which the seed germination practically ceased to occur. The effect of extracts and leachates was studied on radicle and plumule elongation over 96 h duration. The plumule and radicle elongation was averaged on the basis of ten measurements. The effect of the extract/leachate was also studied on seed germination and plumule and radicle growth of *E. riparium*.

Experiment 2: Growth of Galinsoga *spp. as affected by* E. riparium *extract and leachate*

Four day old five seedlings of each of the *Galinsoga* spp., with fully expanded cotyledonary leaves were separately transplanted on 20 July 1980 in each of the 36 plastic pots (21 cm diam. with a basal hole for drainage) containing 4 kg sandy loam soil. The pots were separated in four lots for application of 1% and 5% extracts and leachates. There were three replicates and three harvests for each of the four treatments. The aqueous extracts/leachates were prepared as described in Experiment 1. Three hundred ml of each extract/leachate was supplied to each pot of the respective treatments at 3-days interval from the date of transplantation. Nine control pots where 300 ml of distilled water was supplied, were kept for each test species. No nutrients were added to the pot soils exogeneously. The harvests were taken after 5, 10 and 15 weeks from the date of transplantation to determine the leaf area and number of capitula and seeds per plant. The dry matter production was determined after drying the plant material in an oven at 70°C for 48 h.

Experiment 3: Effect of litter extract and litter bed on growth of Galinsoga *spp.*

Experimental design to evaluate the effect of the litter extract was similar to that of Experiment 2. Litter bed was prepared in pots containing soil overlain with 15 g of litter powder. Four day old 5 seedlings were transplanted in each pot on 28 July 1980. The plants from the three pots per treatment were harvested after 15 weeks from the transplantation date and the relevant growth parameters like leaf area, seed output and biomass production were measured.

Experiment 4: Effect of E. riparium *on* Galinsoga *spp. and soil microbial populations*

Twenty pots were filled with equal quantity of sandy loam soil. Two plants of *E. riparium* each having four leaves were transplanted in each of the 10 pots in March 1981. The other set of 10 pots contained no plant (control). After 4 months of growth *E. riparium* plants were uprooted from the pot soil and were left to dry and decompose in the pots for one month,

after which the undecomposed plant material was removed from the pots. Hundred seeds of each of the two *Galinsoga* species were separately sown in the treated and control pots in August 1981. Seeds were also sown in pots filled with soil from the natural habitats of *E. riparium.* The seed germination was recorded after 15 days from sowing while the observations on number of survivors and dry matter production were made after 95 days from sowing. Before seed sowing, the soil samples were collected from each pot and the soil microbial populations were estimated by 'dilution-plate technique' using 'nutrient agar media' for bacteria and 'Martin Rose-Bengal media' for fungi and 'starch caesin agar media' for actinomycetes.

Experiment 5: Effect of decomposing litter of E. riparium *on* Galinsoga *spp. and soil microbial populations*

Fresh plant litter was collected from the field densely infested with *E. riparium.* Effect of litter was studied by mixing the litter to the soil at the rate of 500 g/m^2 which is equivalent to the quantity of litter present on per m^2 area in the field. Both species of *Galinsoga* are characterised by having more than one seedling cohorts emerging at different time period in nature[26] and so, the seedlings are likely to be affected by the litter in different stages of decomposition. To simulate this, the litter was added to the pot soil before 2, 10 and 20 weeks from the start of experiment. Fifty seeds per pot of each of the two test species were separately sown in the treated soil on 20 August 1981. The corresponding controls, where no litter was added were also kept for comparison. Three replicates per treatment were maintained. The observation on seed germination was recorded after 15 days from the sowing date and number of survivors and dry matter yield were determined after 95 days. Before seed sowing, the soil samples collected from the pots were analysed for microbial population by the method described in Experiment 4. The organic matter content of the soil was determined by rapid titration method[27] and pH by a digital pH meter.

Experiment 6: Growth of Aspergillus flavus *and* Trichoderma viride *as affected by* E. riparium *litter extract*

Pure cultures of *A. flavus* and *T. viride* were maintained on 'potato-dextrose agar media'. In order to see the effect of litter extract on growth of the two fungi, two sets of Petri dishes (10 cm diam.), the first set containing PDA media incorporated with milipore (pore size = 0.2 μm) filtered *E. riparium* litter extract which gave 2% and 6% concentrations, and the second set (control) where extract was substituted with equal amount of sterilized distilled water, were maintained. pH of the media in both sets was 6.2. The colony discs of 3 mm diameter, cut with the help of cark borer from pure culture of the two species, were separately innoculated (one disc per Petri dish) on media. All the Petri dishes were incubated at 25°C. The first diameter measurement of the colony discs was done after 2 days of innoculation and the subsequent observations were made at 24 h interval over the next 5 days. Each treatment was replicated five times.

Statistical treatment of the data

The LSD and SE values were calculated to find out whether the differences were significant due to the treatments.

Results

The seed germination and radicle and plumule growth of both the test species of *Galinsoga* were considerably inhibited by extracts and leachates of *E. riparium* (Table 1) and the inhibitory action was correlated with the concentration of extracts/leachates. The two species responded differentially to aqueous extract; in case of *G.*

Table 1. Seed germination and radicle and plumule growth inhibition (%) of *G. ciliata* and *G. parviflora* caused by leaf extract, leachate and litter extract of *E. riparium*

	Leaf extract		Leaf leachate		Litter extract		LSD from control $p = 0.05$
	1%	5%	1%	5%	1%	5%	
G. ciliata							
Seed germination	20.9	61.8	4.3	12.4	18.7	43.9	8.5
Radicle growth	53.8	67.3	14.7	39.4	5.6	35.8	12.8
Plumule growth	53.7	65.5	19.2	28.5	12.7	26.9	13.6
G. parviflora							
Seed germination	16.1	33.5	6.9	18.5	20.3	38.9	9.4
Radicle growth	1.2	34.1	19.0	29.7	12.3	48.3	10.7
Plumule growth	21.3	31.1	6.9	21.5	22.6	43.8	12.6

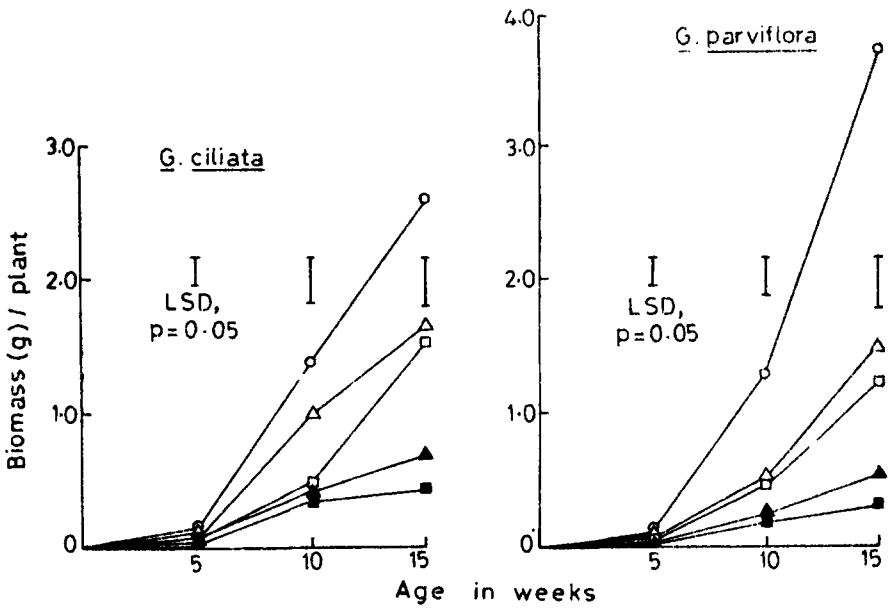

Fig. 1. Growth of *G. ciliata* and *G. parviflora* as affected by varying concentrations of leaf extract and leaf leachate of *E. riparium*. Control (○–○), 1% (□–□) and 5% (■–■) extract concentration and 1% (△–△) and 5% (▲–▲) leachate concentration.

ciliata the leaf extract caused relatively greater inhibition while in *G. parviflora* maximum inhibition was caused by the litter extract. The seed germination and radicle and plumule growth of *E. riparium* were, however, not inhibited by its extracts/leachates.

Although the growth of both test species was adversely affected by the application of leaf extract/leachate of *E. riparium* to the pot soil (Fig. 1), *G. parviflora* was affected more. The leaf area, and number

Table 2. Leaf area (cm^2) and reproductive behaviour of *G. ciliata* and *G. parviflora* as affected by leaf extract and leachate of *E. riparium* (± S.E.)

		Leaf extract		Leaf leachate	
	Control	1%	5%	1%	5%
G. ciliata					
Leaf area/plant	360.3 ± 28.4	308.0 ± 30.2	167.8 ± 36.3	310.0 ± 31.4	192.8 ± 40.7
No. of capitula/plant	161.6 ± 28.4	78.7 ± 16.3	28.3 ± 9.2	103.8 ± 20.4	65.4 ± 11.9
No. of seeds/capitulum	18.9 ± 1.6	19.2 ± 1.3	16.5 ± 2.1	19.0 ± 0.9	18.3 ± 1.8
No. of seeds/plant	3055 ± 601	1512 ± 208	466 ± 89	1972 ± 401	1196 ± 215
G. parviflora					
Leaf area/plant	406.6 ± 80.3	226.0 ± 72.9	133.7 ± 10.8	228.0 ± 70.3	135.0 ± 12.1
No. of capitula/plant	99.3 ± 13.3	45.0 ± 12.1	11.3 ± 4.3	59.9 ± 14.3	16.4 ± 2.4
No. of seeds/capitulum	25.0 ± 2.7	24.0 ± 1.2	24.2 ± 1.6	26.1 ± 2.1	23.9 ± 1.6
No. of seeds/plant	2481 ± 702	1080 ± 103	272 ± 48	1557 ± 308	401 ± 82

Table 3. Leaf area (cm^2), reproductive behaviour and dry weight (mg) of *G. ciliata* and *G. parviflora* as affected by litter extract and litter bed of *E. riparium* (± S.E.)

	Control	Litter extract 1%	Litter extract 5%	Litter bed
G. ciliata				
Leaf area/plant	272.8 ± 24.3	198.3 ± 18.6	50.2 ± 10.8	214.3 ± 38.6
No. of capitula/plant	48.2 ± 12.3	27.4 ± 4.6	10.4 ± 2.3	14.8 ± 4.1
No. of seeds/capitulum	17.4 ± 1.3	18.1 ± 1.6	17.9 ± 1.9	18.3 ± 2.1
No. of seeds/plant	838 ± 212	496 ± 93.1	186 ± 26.3	271 ± 39.4
Dry weight/plant	812 ± 38.3	521 ± 22.6	255 ± 20.2	581 ± 27.5
G. parviflora				
Leaf area/plant	342.0 ± 20.6	173.0 ± 15.2	98.3 ± 9.6	122.5 ± 12.3
No. of capitula/plant	53.0 ± 13.1	23.8 ± 5.0	12.8 ± 4.3	9.6 ± 3.3
No. of seeds/capitulum	24.3 ± 1.6	24.7 ± 1.6	25.1 ± 2.1	24.7 ± 2.1
No. of seeds/plant	1288 ± 210	588 ± 63	320 ± 49	237 ± 31
Dry weight/plant	1804 ± 41.3	752 ± 31.6	374 ± 16.8	338 ± 17.3

Table 4. The inhibitory effect (%) of leaf extract, leachate and litter extract and litter bed of *E. riparium* on leaf area, seed output and biomass of *G. ciliata* and *G. parviflora*

	Leaf extract		Leaf leachate		Litter extract			
	1%	5%	1%	5%	1%	5%	Litter bed	LSD from control $p = 0.05$
G. ciliata								
Leaf area	14.4	53.5	13.9	46.5	27.3	81.6	21.5	22.2
Seed output	50.5	84.7	35.5	60.8	40.8	77.8	67.6	37.3
Root biomass	25.3	58.0	13.5	40.2	50.0	77.0	62.5	30.1
Shoot biomass	44.0	80.0	30.5	59.0	39.2	70.0	34.5	35.2
G. parviflora								
Leaf area	44.5	67.1	44.0	66.8	49.4	71.3	64.2	44.7
Seed output	56.5	88.8	37.2	83.8	54.4	75.2	81.6	38.3
Root biomass	50.0	68.2	24.0	53.8	57.0	81.5	78.0	26.5
Shoot biomass	59.6	89.3	42.0	64.1	54.2	77.0	78.2	44.2

of capitula and seeds per plant of the two species were substantially reduced due to extract/leachate application (Table 2), but the number of seeds per capitulum remained unchanged. Similar reduction in growth performance of the test species was observed due to the litter extract application and amendment of soil with litter (Table 3). The leaf extract caused most inhibition, followed by the litter extract and leaf leachate (Table 4). The shoot growth of the test species was relatively more affected by the leaf extract and leaf leachate while the root growth was more affected by the litter extract and litter bed.

Seed germination, survival and growth and reproductive allocation

Table 5. Seed germination, survivorship, dry matter yield and reproductive allocation of *G. ciliata* and *G. parviflora* as affected by the growth of *E. riparium*

Nature of pot soil	Seed germination %	Survivorship %	Yield/pot (g)	Reproductive allocation %
G. ciliata				
Soil from natural habitat of *E. riparium*	80.0	47.8	4.2	10.7
Soil from pots containing *E. riparium*	67.5	43.5	4.0	12.8
Soil from pots devoid of *E. riparium*	86.3	58.7	5.2	13.7
LSD, $p = 0.05$	18.6	5.5	1.0	3.9
G. parviflora				
Soil from natural habitat of *E. riparium*	76.6	36.5	4.9	7.1
Soil from pots containing *E. riparium*	72.6	39.1	4.9	8.8
Soil from pots devoid of *E. riparium*	89.7	43.5	6.08	12.1
LSD, $p = 0.05$	12.2	2.6	0.92	2.9

of both species were reduced when the plants were grown in soil collected from *E. riparium* stand (Table 5). *G. parviflora* was more adversely affected than *G. ciliata.*

The populations of fungi, bacteria and actinomycetes in the soil were reduced to the extent of 25% due to presence of *E. riparium* (Fig. 2). The qualitative analysis of fungal flora showed the contrasting behaviour of two fungal species, *T. viride* and *A. flavus,* the former being more abundant in soil collected from *E. riparium* stand and the latter being absent from such soil (Table 6).

The litter of *E. riparium* in different stages of decomposition in the soil caused reduction in seed germination, number of survivors and reproductive performance of both species of *Galinsoga* (Table 7). The litter in early stage of decomposition inhibited the plant growth more adversely than that in the advanced stage of decomposition. The addition of the litter to the pot soil two weeks before seed sowing resulted in an increased shoot/root ratio, especially in *G. ciliata.*

Soil microbial population estimated after 2 weeks from the date of litter addition was also reduced (Fig. 3). Bacterial population was much more affected than the populations of fungi and actinomycetes. Later on, as the litter decomposition proceeded further, a sharp increase in microbe population and organic matter content of soil was observed. Soil pH did not vary much (range 5.2–5.8) in different treatments. The qualitative analysis of fungal flora revealed an

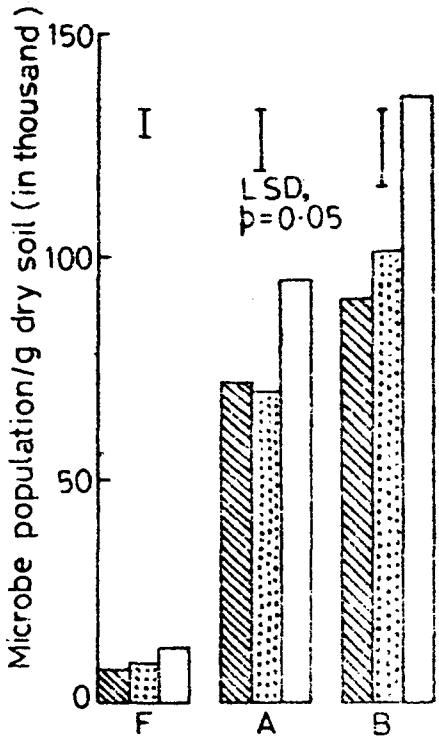

Fig. 2. Soil microbial populations (F. Fungi; A. actinomycetes and B. bacteria) as affected by the growth of *E. riparium*. Hatched columns for soil collected from natural habitats of *E. riparium*, dotted columns for soil collected from pots containing *E. riparium* plants and open columns for soil collected from pots devoid of *E. riparium* plants.

Table 6. Quantitative distribution of soil fungi as affected by the growth of *E. riparium* (colonies in thousand per g dry soil)

Fungal species	Soil from natural habitat of *E. riparium*	Soil from pots containing *E. riparium*	Soil from pots devoid of *E. riparium*	LSD $p = 0.05$
Trichoderma viride	2.67	2.85	0.84	1.01
Aspergillus flavus	0.0	0.0	3.50	0.00
Penicillium chrysogenum	1.68	1.67	2.23	0.93
Aspergillus niger	0.39	0.32	0.50	0.02
Mucor hiemalis	0.54	0.18	1.30	0.69
M. circinelloides	0.00	0.33	0.00	0.0
Monilia humicola	0.67	1.66	1.00	0.63
Fusarium oxysporum	0.33	0.67	0.50	0.21
Cladosporium herbarum	0.38	0.67	0.50	0.14
Aureobasidium pullulans	1.33	1.33	1.00	0.39

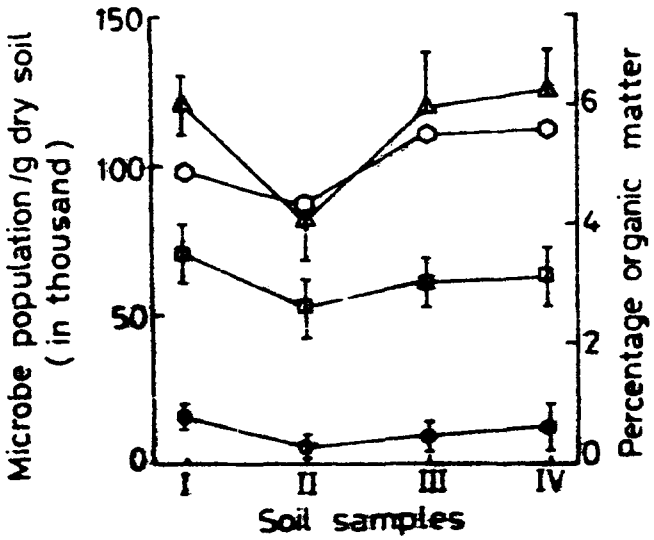

Fig. 3. Changes in microbial populations and organic matter content of the soil due to amendment with decomposing litter of *E. riparium*. Symbols: ○–○ for fungi; □–□ for actinomycetes; △–△ for bacteria and ◇–◇ for organic matter content. Vertical bars show S.E. of means. The soil samples I, II, III and IV refer to the unamended soil, and the soils amended with the litter 2, 10 and 20 weeks before sowing.

Table 7. Seed germination, survivorship, yield, shoot/root ratio and reproductive allocation of *G. ciliata* and *G. parviflora* as affected by decomposing litter of *E. riparium*

Duration of litter decomposition in pot soil	Seed germination %	Survivorship %	Yield/pot (g)	Shoot/root ratio	Reproductive allocation %
G. ciliata					
Control*	75.6	81.1	2.7	11.6	9.6
2 weeks	56.3	44.4	0.7	16.5	4.2
10 weeks	65.6	59.4	1.8	12.5	6.8
20 weeks	65.6	76.1	1.9	13.4	10.6
LSD, $p = 0.05$	14.7	21.4	0.6	2.2	2.6
G. parviflora					
Control*	72.5	71.4	3.1	11.0	6.5
2 weeks	50.7	42.8	0.9	13.5	3.9
10 weeks	76.1	52.3	2.6	13.4	6.7
20 weeks	68.2	61.3	2.3	12.6	6.6
LSD, $p = 0.05$	16.3	19.2	0.5	2.4	1.0

* No addition of litter

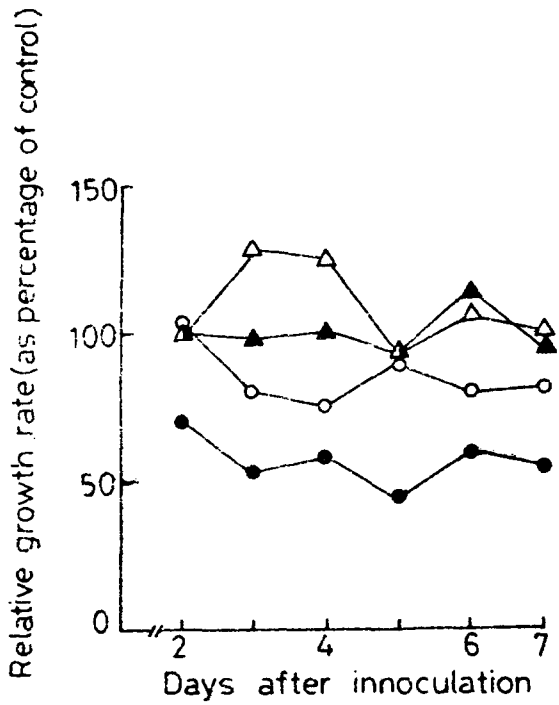

Fig. 4. Relative growth rate of *T. viride* (△) and *A. flavus* (○) as affected by varying concentrations of litter extract of *E. riparium* (open symbols represent the low concentration and solid symbols the high concentration of the litter extract.

Table 8. Quantitative distribution of soil fungi as affected by the soil amendment with decomposing litter of *E. riparium* (colonies in thousand per g dry soil)

Fungal species	Decomposition of litter (weeks) Control*	2	10	20	LSD $p = 0.05$
Alternaria humicola	0.67	0.33	0.67	0.42	0.28
Aspergillus flavus	5.5	2.03	1.98	2.33	1.03
A. niger	2.0	0.67	0.34	1.33	1.06
Cladosporium herbarum	1.21	0.33	0.33	1.00	0.79
Mucor hiemalis	0.33	0.0	0.0	0.0	0.0
Mycelia sterilia	1.40	0.33	0.69	1.67	1.06
Penicillium chrysogenum	0.67	1.00	1.33	1.30	0.67
Pythium elongatum	2.0	0.0	0.37	0.0	0.0
Trichoderma viride	0.66	1.83	2.24	3.66	1.06

* No addition of litter.

increase in *T. viride* and decrease in *A. flavus* population in the amended soil (Table 8). Similar contrasting behaviour of these two fungi was observed in response to the addition of the litter extract in pure culture. Unlike the high concentration of the litter extract, the lower concentration enhanced the growth of *T. viride*, while in the case of *A. flavus* both the concentrations were inhibitory (Fig. 4).

Discussion

Not only the germination of seeds of the *Galinsoga* spp. soaked with extracts and leachates of *E. riparium* was reduced but the growth of plumule and radicle emerging out of such seeds was also inhibited. Suppressed growth of the seedlings caused due to inhibitory action of *E. riparium* might render the *Galinsoga* species still weaker with regard to competition against *E. riparium.* Such an argument finds support from Lockerman and Putnam[8]. As a consequence, the competition and allelopathic effects of *E. riparium* on the *Galinsoga* spp. play a crucial role in population regulation of the latter. The contrasting behaviour of the two fungal species *viz.; T. viride* and *A. flavus,* in response to allelochemics confirms the contention of Akhtar *et al.*[1] and Whittaker[29] that allelopathic action depends on the nature of test species and concentration of allelochemics. Further, the absence of auto-toxicity in *E. riparium* parallels the observations of several other workers[15,17] that the allelopathic effects depend on the strain of donor and recipient plant species. Not only this, the shoot and root of the test species exhibit differential susceptibility to the plant extracts. For example, the root growth was much more affected by the litter extract, and soil amended with the litter of *E. riparium* as compared to shoot growth whereas the latter was relatively more adversely affected by the leaf extract. This is in agreement with the observations of Tripathi *et al.*[25].

Like other allelopathic plants[3,6,13], *E. riparium* induced soil toxicity by releasing toxins through leaching during its active growth and during the decay of litter. As a result, the seed germination and growth of the *Galinsoga* species were reduced considerably when they were grown either in soil collected from natural habitats infested with *E. riparium* or in experimental pots containing the soil amended with the litter of *E. riparium.* Besides, the microbial population of these soils was also much reduced (Figs. 2 and 3). The reduction in growth of the test species and soil microbial population under the influence of *E. riparium* highlights the importance of allelopathy in population regulation of the species as has also been argued by Whittaker[28].

The growth of the *Galinsoga* spp. and microbial population increased with increase in organic matter content of the soil, especially in the treatment where the litter was added to the pot soil 20 weeks prior to seed sowing (Fig. 3). This is supported by an earlier observation[23] where the litter of *Artemisia tridentata* in early decomposition stage retarded seed germination and seedling growth of several grasses,

while four weeks after germination it stimulated the growth. Increase in microbial population with increased organic matter in soil has been reported by several workers[7,11]. The decreased soil microbial population in response to allelochemics might change chemical properties of the soil system. For example, Rice[19,20] and Lodhi[10] observed inhibition of nitrification in grassland and forest community respectively due to presence of allelochemics.

Interestingly enough, while the seed germination and growth of the test species were strongly inhibited by *E. riparium* in bio-assay tests, they do occur in nature as associates of *E. riparium* although in poor density[18]. This might be either due to buffering action of other plant species growing in the vicinity and/or due to difference between the concentrations of allelochemics in nature and in the experiment. Heavy rainfall (ca 2500 mm per year) received in Meghalaya might be having a great diluting effect on the allelochemics produced by *E. riparium.* Thus, some plant species may presumably escape unaffected by allelochemics produced by *E. riparium* and may not show the same extent of inhibition as observed in control conditions[19]. del Moral and Cates[2] and Stowe[24] have also observed a weak correlation between field distribution of a plant species and the bio-assay tests pertaining to allelopathic effects.

The results highlight the importance of allelopathy as a potent factor of population regulation. The bio-assay tests suggest that certain growth inhibitors might be present in *E. riparium,* which have controlling influence on soil microbes and seed germination and radicle and plumule growth of the two *Galinsoga* spp. These chemical substances also regulate the weed seed populations by reducing the seed output per plant. Thus the study provides an interesting example where a weed (*E. riparium* in the present instance) producing allelochemics contributes to the population regulation of the other two weed species.

The exact nature and mechanism of action of the allelochemics produced by *E. riparium* are yet to be understood.

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