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Release of phytotoxins by decomposing roots of Pennisetum typhoides (Burm. f.) Staff et Hubb., their effect on soil fungi and succeeding crops

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The roots of *Pennisetum typhoides* decomposing in normal field conditions, in sterilized soil inoculated with 15 rhizosphere fungi and in field soil maintained at various moisture levels produced vanillic acid, 3-4-dihydroxy benzoic acid and hydroxy cinnamic acid. These acids proved toxic to the rhizosphere fungi and seeds and seedlings of certain crop plants. Out of 15 rhizosphere fungal species inoculated to the soil only 6 could induce the release of toxins, moreover, the phytotoxic substances were detected from the washing of the roots collected only on the 30th day. The moisture range which showed liberation of toxins was 20-70 per cent. The time of liberation of acids in different sets varied. These were, however, frequently liberated from washings collected from roots decomposed for 15, 30 and 45 days.

INTRODUCTION

The first carefully conducted experiments by Schreiner and Reed (1908), Schreiner and Sullivan (1909), Schreiner and Shorey (1909 and 1910) and Skinner (1918) indicated the release of phytotoxic substances by decomposing plant parts in soil. Collison (1925), Doren (1928), Loehwing (1937), Benedict (1941), Cochrane (1948) and McCalla and Duley (1950), supported the findings of the above workers. Bonner (1950) and Borner (1960) presented comprehensive reviews on this aspect of soil microbiology. The investigations Patrick (1955, 1971) Patrick and Koch (1958), Patrick etal. (1964), Toussoun and Patrick (1963) and Latham and Watson (1966) established the validity of phytotoxins. The works mentioned above are mainly restricted to winter crops and this prompted me to conduct such studies with *Pennisetum typhoides* (Burm. f.) Stapf. et Hubb., a main rainy season crop of the tropics, raised on a large acreage in Indian agriculture.

MATERIALS AND METHODS

Pennisetum typhoides (Burm. f.) Stapf. et Hubb., a widely cultivated crop with well developed root system, was raised in the experimental plot situated on the Campus of the University of Gorakhpur, India. The crop was harvested in the month of November, 1971 and aerial parts were removed from the plot. The roots were then asceptically sampled from crown (RC), middle (RM) and disal (RD) regions once in a month till their total decomposition in the field. The root surface washings of the above stated regions were prepared as described by Kanaujia (1973) and used for further studies. The washings were used for the detection of phytotoxins by methods suggested by Smith (1960). The germination of 10 frequently isolated rhizosphere fungi, viz., *Rhizopus nigricans, Mucor hiemalis, Aspergillus fumigatus, A. flavus, A. niger, A. aculeatus, A. terreus, Cladosporium herbarum, Paecilomyces fusisporus* and *Curvularia lunata* was also tested in the root-washings by the hanging drop method (Table 4). The fungal population per gramme dry soil of the respective regions was determined. The moisture content and pH of soil from three different regions was assayed by method described by Piper (1966). The celluloses, hemicelluloses and lignins of the decomposing roots were determined by the method suggested by Peach and Tracey (1955).

The production of phytotoxins by decomposing roots in the presence of certain rhizosphere fungi, viz., *Rhizopus nigricans, Mucor hiemalis, Aspergillus fumigatus, A. flavus, A. niger, A. aculeatus, A. terreus, Trichoderma viride, Penicillium chrysogenum, Cladosporium herbarum, C. epiphyllum, Paecilomyces fusisporus, Fusarium nivale, F. oxysporum* and white sterile fungus was studied (Table 5). The roots collected after harvesting were thorougly washed, dried at 105°C for 24 hours in a hot air oven, cooled to room temperature and weighed into 1 g lundles. Dried roots were then packed into 8 × 5 cm sterilized nylon bags. Five such bundles were burried in 1 kg pot soil which had been inoculated with the above-mentioned fungi a month earlier and incubated. The soil was sterilized at 15 lbs pressure for two hours in a steam sterilizer before the isolates were added to it. The earthen pots used in this experiment were surface sterilized with 0.1% $HgCl_2$ aqueous solution, washed thorougly with distilled water, dried and finally were internally coated with wax. Three replicates were maintained for each fungus. In the control set the sterilized roots were buried in sterilized soil. The pots after burial of the roots were incubated at room temperature (20 - 27°C) for 15 days. For the collection of sufficient root washing the sterilized roots were buried at rate of 500 g per pot containing 10 kg soil for each fungus. The moisture content of the soil was adjusted to 25 ± 2 per cent by adding extra sterilized water at regular intervals. After 15 days one bundle with adhered soil was gently taken out from each pot by sterilized forceps. The roots with soil were transferred to

250 ml sterile conical flasks containing 100 ml double sterilized distilled water. The flasks were handshaken throughly for 15 minutes and the water was filtered. The filtrate was centrifuged at 5000 rpm for 10 minutes and filtered again. This filtrate was designated as root-washing and was used for the detection of phytotoxins, germination of seeds of *Pisum sativum*, *Lens esculentus*, *Brassica campestris*, *B. nigra*, *Triticum aestivum* and *Hordeum vulgare* and of 14 rhizosphere fungi stated earlier. The fungal species adhering to the roots were also determined on the basis of per gramme dry soil.

Production of phytotoxins by roots separately decomposing in the field at 10, 20, 30, 40, 50, 60, 70 and 80 per cent moisture levels was studied by methods described by K a n a u j i a (1973). Freshly dug soil from 24 different places of the field was collected from 0,5 cm to 30 cm depth and was mixed together. This was filled (at the rate of 1 kg per pot) in newly prepared earthen pots which were surface sterilized and washed with sterilized dilstilled water, dried and coated internally with wax. Eight groups each containing 3 pots were made. The moisture content of 1 - 8 groups (sets) was adjusted to 10, 20, 30, 40, 50, 60, 70 and 80 per cent. Freshly harvested roots in the month of December, 1971, were washed, dried, weighed, packed into nylon bags and buried in the soil of the pots as described in the previous case. The pots were covered with polyethylene sacs to avoid excessive evoporation of water from the soil surface. Extra sterilized distilled water was added to the soil at regular intervals to maintain the moisture status of the soil to the desired level $\pm$ 2%. The pots were incubated in the field. The sampling of the roots and preparation of the root-washings were done as in the previous case. The effect of root-washings collected from the decomposing roots kept at different moisture levels on seed and seedlings of six crop plants was also studied as described in the precious case. The effect of root-washing on 14 rhizosphere fungi (same as in the previous experiment) was also studied. The fungal population of soil associated with the roots was calculated on the basis of per gramme dry soil.

RESULTS

a – Production of phytotoxins by roots decomposing in normal field conditions

As evident from Figure 1, root-washings from RC, RM and RD regions exhibited the production of vanillic acid and 3 - 4-dihydroxy benzoic acid in the month of March and April. The two acids were recognized in the washings collected from RM and RD regions respectively. 3 - 4-dihydroxy benzoic acid from RC and vanillic acid from RM and RD regions were detected in the washings collected in the month of April.

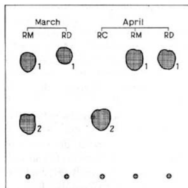

Fig. 1. Detection of phytotoxins in the root-washings of crown (RC), middle (RM) and distal regions (RD) of *Pennisetum typhoides*

1 – vanillic acid; *2* – 3-4-dihydroxy benzoic acid

b – Production of phytotoxins by decomposing roots in soil separately inoculated with certain rhizosphere fungi

Out of 15 sets separately inoculated with rhizosphere fungi, only 6, that is those inoculated with *Mucor hiemalis*, *Aspergillus terreus*, *A. niger*, *Penicillium chrysogenum*, *Paecilomyces fusisporus* and *Trichoderma viride* contained phytotoxins. Vanillic acid was commonly detected in all the six sets mentioned above

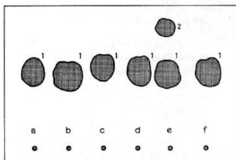

Fig. 2. Detection of phytotoxins in the root-washings of *Pennisetum typhoides* decomposing (30th day) separately in the presence of certain rhizosphere fungi

a – *Mucor hiemalis*, *b* – *Aspergillus niger*, *c* – *A. terreus*, *d* – *Penicillium chrysogenum*, *e* – *Paecilomyces fusisporus*, *f* – *Trichoderma viride*; *1* – vanillic acid, *2* – hydrocinnamic acid

whereas hydro-cinnamic acid was detected in addition in the set inoculated with *Paecilomyces fusisporus* on 30th day of root decomposition. On 15th, 45th, 60th and 75th day none of the root-washings exhibited any toxin (Fig. 2).

c – Production of phytotoxins by decomposing roots in field soil maintained at different moisture levels

It was noticed that all the sets did not produce the phytotoxins and in those which produced them, the time of liberation was not the same. The range of moisture level within which toxins were detected was 20 - 70 per cent. On the 15th day, vanillic acid was identified in sets kept at 50 and 60 per cent moisture content. On the 30th day, the above acid was detected in 40, 50 and 60 per cent sets and 3 - 4-dihydroxy benzoic acid in 40, 60 and 70 per cent sets. On the 45th day, vanillic acid alone was chromatogrammed from the root-washing of the sets kept 20, 30 and 40% moisture levels. On the 60th and 70th day no phytotoxin was detected from any set (Fig. 3).

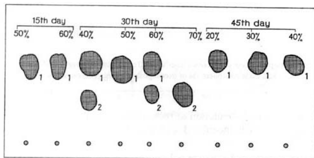

Fig. 3. Detection of phytotoxins in the root-washings of *Pennisetum typhoides* decomposed at different moisture levels for 15, 30 and 45 days

1 – vanillic acid, *2* – 3-4-dihydroxy benzoic acid

d – Fungal population of Crown, Middle and Distal regions of the roots

During January to April the maximum fungal population was recorded in the crown region. Later on from April to June, it generally was the highest in the distal region. A low population was observed in the middle and distal regions in

March and in all the regions in the month of April. The population in the nonrhizosphere region was at all times lower than in the corresponding root regions (Fig. 4).

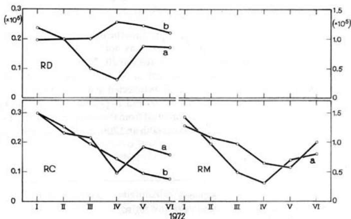

Fig. 4. Fungal population of crown (RC), middle (RM) and distal (RD) regions of rhizosphere (*a*) and nonrhizosphere (*b*) of decomposing *Pennisetum typhoides*

e – Fungal population at root surface decomposing in soil, separately inoculated with selected rhizosphere fungi

No regular pattern of fungal population in different sets was obtained. On the 15th day, an appreciably higher population was obtained in sets inoculated with *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum*, *Cladosporium* and *Paecilomyces fusisporus*. On the 30th day, the population in *Mucor hiemalis*, *Aspergillus terreus*, *A. niger*, *Trichoderma viride*, *Penicillium chrysogenum* and *Paecilomyces fusisporus* increased considerably. In the remaining sets it increased. On the 45th day, the population was low in sets inoculated with *Aspergillus fumigatus* and *Trichoderma*. On the other hand, in the majority of the cases it showed an increasing tendency. On the 60th day of root decomposition and also on the 75th day no regular pattern was noticed except for a decrease in all the sets (Fig. 5).

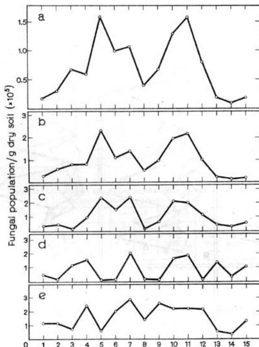

Fig. 5. Population of 15 rhizosphere fungi in sterile soil amended separately with their cultures
a – 25 II 1972, *b* – 10 II 1972, *c* – 26 I 1972, *d* – 11 I 1972, *e* – 27 XII 1971
1 – *Rhizopus nigricans*, *2* – *Mucor hiemalis*, *3* – *Aspergillus fumigatus*, *4* – *A. flavus*, *5* – *A. niger*, *6* – *A. aculeatus*, *7* – *A. terreus*, *8* – *Trichoderma viride*, *9* – *Penicillium chrysogenum*, *10* – *Cladosporium herbarum*, *11* – *C. epiphyllum*, *12* – *Paecilomyces fusisporus*, *13* – *Fusarium nivale*, *14* – *F. oxysporum*, *15* – a white sterile fungus

f – Fungal population associated with the roots decomposing at different moisture levels

The fungal population was always low in the 10% set. It showed an increasing tendency with an increase in moisture content, resulting in the highest population generally in 20% set. The sudden decrease in population of the 40% set was observed on all the sampling dates. Least variation in fungal population was recorded in soil kept at 10% whereas in the 20% set it showed an increasing

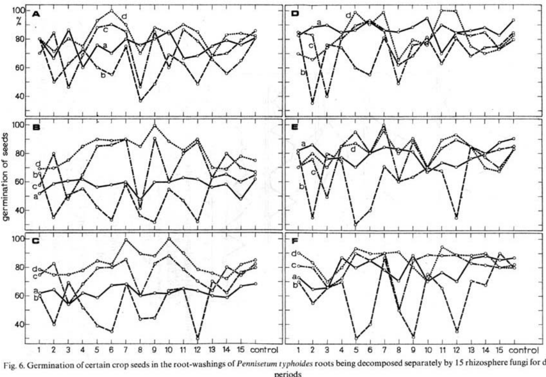

Fig. 6. Germination of certain crop seeds in the root-washings of *Pennisetum typhoides* roots being decomposed separately by 15 rhizosphere fungi for different periods

a – 27 XII 1971, *b* – 11 I 1972, *c* – 26 I 1972, *d* – 10 II 1972; 1-15 – see Fig. 5

A – *Brassica campestris*, *B* – *Lens esculentus*, *C* – *Pisum sativum*, *D* – *Brassica nigra*, *E* – *Hordeum vulgare*, *F* – *Triticum aestivum*

tendency from the 15th to 75th day of decomposition of roots. In the remaining sets a decreasing level from beginning to the end of the experiment was obtained (Table 1). Variation in the fungal population was significant and was caused partly by amendment and partly by root-washings of different age (Table 2).

Table 1

Fungal population /X 10^5/ of plot soil mainstained at different moisture levels at various decomposition stages of the root

Sampling period year 1972	Moisture levels /%/							
	10	20	30	40	50	60	70	80
1.I	0.6	1.0	1.7	0.9	0.6	0.2	0.08	0.07
15.I	0.4	1.8	1.5	0.5	0.2	0.08	0.03	0.02
30.I	0.6	1.9	1.2	0.3	0.06	0.02	0.02	0.01
14.II	0.5	2.1	1.1	0.2	0.05	0.03	0.02	0.01
29.II	0.6	2.4	0.9	0.10	0.04	0.03	0.01	0.01

Table 2

Analysis of variance for fungal population of plots maintained at varying moisture levels

Variation due to	SS	df	Variance	F-calculated	F-tabulated	
Amendment	15.62	7	2.23	74.3*	2.36	3.36
Root-washings of different age	1.02	4	0.25	8.3*	2.71	4.07
Exp. Error	1.07	28	0.03			

* Significant at 1% level

g – Effect of washings of roots being decomposed by certain rhizosphere fungi on seed germination

Generally no difference in the germination of seeds was noticed in washings of the roots decomposed for 15 days. A slight inhibitory effect, however, was observed in the case of the set inoculated with *Cladosporium* amended set. The germination of *Brassica compestris, Hordeum vulgare* seeds was slightly promoted by the root washings collected from the sets inoculated with fungi given in paranthesis. On the 30th day, washings from *Mucor hiemalis, Aspergillus terreus, A. niger, Trichoderma viride, Penicillium chrysogenum* and *Paecilomyces fusisporus* appreciably lowered the germination of all the 6 types of seeds whereas on the 45th day, root-washings of sets inoculated with *Aspergillus fumigatus* and *Trichoderma viride* caused a lowering of germination of all the seed types. Washings collected on the 60th and 75th day of root decomposition enhanced the germination of all the seeds (Fig. 6). In the majority of the cases the variation in seed germination caused by root-washings decomposed for different periods was found to be significant when data were statistically processed (Table 3).

Table 3

Analysis of variance for the germination of certain seeds in the washings of decomposing roots amended with certain fungal isolates

Seeds	Variation due to	SS	df	Variance	F-Calculated	F-Tabulated	
Pisum sativum	Amendment	2258.1	15	150.54	1.5	1.87	2.42
	Decomposition age	7593.6	3	2531.2	226.84*	2.81	4.24
	Exp. Error	4244.5	45	94.3			
Lens esculentus	Amendment	1615.0	15	107.6	0.74	1.87	2.42
	Dec. age	10283.1	3	3427.7	23.72*	2.81	4.24
	Exp. Error	6501.9	45	144.49			
Brassica compestris	Amendment	2373.2	15	158.2	0.49	1.87	2.42
	Dec. age	26188.6	3	8729.53	27.43*	2.81	4.24
	Exp. Error	14320.0	45	318.22			
Brassica nigra	Amendment	3417.4	15	227.8	13.4*	1.87	2.42
	Dec. age	3546.2	3	1182.1	69.5*	2.81	4.24
	Exp. Error	7648.2	45	16.99			
Triticum aestivum	Amendment	2022.7	15	134.8	0.424	1.87	2.42
	Dec. age	2571.3	3	857.1	2.69	2.81	4.24
	Exp. Error	14292.5	45	318.6			
Hordeum vulgare	Amendment	24595.6	15	1639.7	1.194	1.87	2.42
	Dec. age	4940.7	3	1646.9	1.199	2.81	4.24
	Exp. Error	61782.3	45	1372.9			

* significant at 1% level

h – Effect of washings of roots being decomposed in field soil at different moisture levels on seed germination and seddling growth

The root-washings from 50 and 60 per cent sets collected on the 15th day appreciably reduced the seed germination. In the remaining cases, however, the inhibitory property was very mild. On the 30th day, the germination of all the seeds was very low in the root-washings collected from 40, 50, 60 and 70% moisture levels. The washings from remaining 10% sets also exerted an adverse effect on seed germination, however, the effect was mild. The washing from 40% set was most inhibitory on the 45th day whereas in the remaining sets these showed an inhibitory activity. The germination of all the seeds was generally enhanced by washings on 60th and 70th day of root decomposition. The study of collection of the washings from 60 and 75% moisture status could not be possible due the total decomposition of the roots on 75th day (Fig. 7). The effect of washings on the root and shoot growth of 6 crop plants was in accordance with their respective seed germination (Fig. 8). In the majority of the cases a reduction in the root and shoot was observed. Root curvatures and black patches which appeared here and there on both roots and shoots were observed.

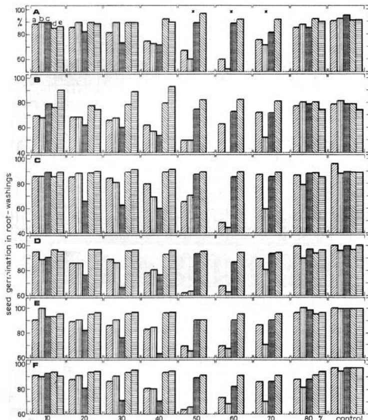

Fig. 7. Germination of six crops in the washings of *Pennisetum typhoides* being decomposed separately in soil at different moisture levels 10 - 80%

a – 1 I 1972, *b* – 15 I 1972, *c* – 30 I 1972, *d* – 14 II 1972, *e* – 29 II 1972

A – *Brassica campestris*, *B* – *Lens esculenta*, *C* – *Pisum sativum*, *D* – *Brassica nigra*, *E* – *Hordeum vulgare*, *F* – *Triticum aestivum*

x – in (A - F) reduced the seedlings growth

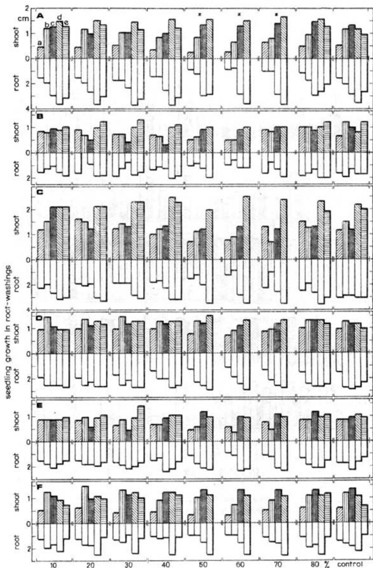

Fig. 8. Seedling growth of six crop plants in the washings of *Pennisetum typhoides* roots being decomposed separately in soil at moisture levels 10 - 80%

Explanations – see Fig. 8

i – Effect of root-washings from RC, RM and RD regions of decomposing roots on rhizosphere fungi

The rhizosphere fungi germinated to varying levels in the root-washings collected from RC, RM and RD regions of roots. The pattern of germination of all the fungi showed an increasing tendency from January to February. In the month of March and April, washings collected from RC, RM and RD regions appreciably lowered the germination of nearly all the test fungi, however, the washings from the latter two regions were more toxic than the former. The germination of the spores of all the test fungi in the succeeding month was enhanced whereas in June the germination in every case was very near to their respective controls (Table 4).

Table 4
Effect of root-washings from crown /RC/, middle /RM/ and distal /RD/ regions of P. typhoides roots on certain root fungi /1-10/

Root-washings of 1972	Test fungal spores									
	1	2	3	4	5	6	7	8	9	10
January										
RC	75	65	56	67	58	73	70	80	55	98
RM	70	60	50	63	60	75	65	75	55	93
RD	70	71	58	68	60	80	68	82	50	98
Control	65	50	45	60	55	70	65	70	40	90
February										
RC	80	68	58	70	60	75	73	88	60	100
RM	73	63	55	65	61	75	68	78	57	95
RD	70	74	63	73	65	83	71	85	58	100
Control	55	47	48	63.	65	70	65	75	45	90
March										
RC	71	60	55	65	61	64	55	53	60	83
RM	30	26	35	40	43	50	41	31	37	60
RD	34	33	39	43	47	60	45	48	43	70
Control	50	42	50	65	60	65	50	50	50	87
April										
RC	47	27	40	51	50	47	40	32	37	75
RM	33	38	39	43	40	47	28	32	38	73
RD	34	33	40	43	47	60	45	48	43	70
Control	50	40	55	65	60	63	50	65	57	80
May										
RC	47	27	40	51	50	47	40	32	67	75
RM	33	38	39	43	40	47	28	32	38	65
RD	30	30	35	45	42	62	47	40	55	65
Control	50	40	55	65	60	63	50	45	57	80
June										
RC	60	40	50	70	73	65	60	55	80	91
RM	61	41	43	63	58	57	55	47	68	70
RD	45	47	50	60	63	53	65	55	60	83
Control	45	47	40	60	55	50	40	40	56	75

1 – Rhizopus nigricans, 2 – Mucor hiemalis, 3 – Aspergillus fumigatus, 4 – A. flavus, 5 – A. niger, 6 – A. aculeatus, 7 – A. terreus, 8 – Cladosporium herbarum, 9 – Paecilomyces fusisporus, 10 – Curvularia lunata; Data express the germination /%/.

j – Effect of washings of roots, separately decomposed by certain fungal isolates on some rhizosphere fungi

The germination of 14 test fungi described on the preceeding pages varied differently in the root-washings of various sets inoculated by fungi. There existed no regular pattern except that the germination in most of the cases was generally higher in the sets where the test fungus itself had been inoculated. On the 15th day, the germination of nearly all the test fungi was approaching their respective controls. On the 30th day, the washings from sets inoculated with *Mucor hiemalis*, *Aspergillus niger*, *A. terreus*, *Penicillium chrysogenum*, *Trichoderma viride* and *Paecilomyces fusisporus* appreciably decreased the germination of test fungal spores. The general trend though was very near to that of the 15th

Table 5

Germination /%/ of certain fungi /1-14/ in the root washings of roots decomposed separately by these fungi on 30th day

Root-washings from the set amended with	Test fungi													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Rhizopus nigricans	70	69	47	51	63	67	60	63	57	58	56	61	58	46
2. Mucor hiemalis	46	56	47	40	37	46	50	37	40	53	56	60	48	40
3. Aspergillus fumigatus	60	55	70	47	57	60	63	60	62	50	51	57	48	42
4. A. flavus	80	56	73	79	70	65	63	72	63	64	68	64	56	47
5. A. terreus	50	53	50	40	60	56	50	53	47	36	42	47	50	30
6. A. niger	40	47	40	56	52	73	62	53	58	65	67	60	40	30
7. A. aculeatus	67	63	47	60	64	67	70	63	52	75	64	48	56	51
8. Trichoderma viride	30	37	42	45	47	41	37	67	53	50	47	40	32	37
9. Penicillium chrysogenum	50	50	60	57	61	47	51	63	70	47	34	47	42	43
10. Cladosporium epiphyllum	73	51	67	47	56	63	60	60	74	76	63	60	40	35
11. C. herbarum	80	50	65	48	53	67	67	70	70	70	87	79	70	57
12. Paecilomyces fusisporus	60	47	31	47	41	48	49	34	37	47	63	76	48	47
13. Fusarium nivale	72	80	40	60	67	67	63	75	79	75	87	80	89	62
14. F. oxysporum	60	75	47	67	70	68	75	73	70	70	80	70	80	83
15. White sterile fungus /W1/.	73	76	65	70	64	76	65	60	68	72	76	79	80	82
Control /Distilled water/	72	68	68	72	67	63	60	76	79	75	75	60	90	90

Table 6

Germination /%/ of certain fungi /1-14/ in the root-washings of roots decomposed separately by these fungi for 45 days

Root-washings from the sets amended with	Test fungi													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Rhizopus nigricans	60	64	63	76	79	69	65	71	77	73	75	46	49	56
2. Mucor hiemalis	63	66	63	70	76	63	60	72	56	69	76	49	50	53
3. Aspergillus fumigatus	40	43	46	43	31	47	37	47	40	50	52	43	46	40
4. A. flavus	52	60	67	79	75	61	62	69	53	65	76	51	53	55
5. A. terreus	57	62	69	72	59	62	56	65	53	66	79	53	52	57
6. A. niger	55	63	70	73	53	69	53	67	52	67	78	55	51	60
7. A. aculeatus	56	66	70	75	61	66	83	56	53	67	73	56	46	47
8. Trichoderma viride	47	42	50	50	43	46	42	70	42	51	61	37	62	47
9. Penicillium chrysogenum	52	63	73	76	58	73	58	67	73	63	70	56	43	47
10. Cladosporium epiphyllum	51	47	76	63	85	80	59	70	57	73	75	56	48	50
11. C. herbarum	62	49	75	69	82	62	60	76	56	66	89	83	86	51
12. Paecilomyces fusisporus	75	52	75	75	81	67	63	70	57	65	70	52	46	53
13. Fusarium nivale	70	73	79	75	79	65	60	73	60	63	76	55	47	53
14. F. oxysporum	67	72	78	77	73	62	60	74	63	62	81	69	46	56
15. White sterile fungus /W1/	63	66	72	75	75	60	61	72	65	68	83	63	52	53
Control	65	63	70	70	68	60	58	68	70	73	73	65	66	69

day. On the 45th day washings from *Trichoderma viride* and *Aspergillus fumigatus* inhibited the germination of all the test fungi. In the remaining cases a promotory effect was observed (Tables 5, 6). On the 60th and 75th day washings from all the sets proved promotive to various test organisms (only data of 30th and 45th days where an adverse effect was observed are represented).

k – celluloses, hemicelluloses and lignins of RC, RM and RD regions of decomposing roots

The cellulose, hemicellulose and lignin components of the roots in RC, RM and RD regions exhibited a regular pattern. Their levels were highest in the month of January, showed a decreasing tendency till June. Comparatively a more pronounced decrease in the amounts of celluloses and hemicelluloses was noticed than that of lignins in all the three root regions. The rapid decomposition of celluloses, hemicelluloses and lignins was observed during March and April (Table 7).

Table 7
Celluloses, hemicelluloses and lignin components of crown /RC/, middle /RM/ and distal /RD/ regions of the roots

Sampling date /1972/	Root-regions	Celluloses /%/	Hemicelluloses /%/	Lignins /%/
January	RC	28.2	15.3	22.0
	RM	21.0	17.0	20.0
	RD	20.0	13.0	20.0
February	RC	21.0	13.0	20.0
	RM	16.0	13.0	19.0
	RD	14.0	11.0	20.0
March	RC	19.0	12.0	20.0
	RM	10.0	11.0	18.0
	RD	11.0	9.0	17.0
April	RC	16.0	10.0	19.0
	RM	7.0	10.0	18.0
	RD	9.0	7.0	15.0
May	RC	15.0	9.0	18.0
	RM	6.0	10.0	17.0
	RD	8.0	5.0	12.0
June	RC	11.0	6.0	17.0
	RM	5.0	8.0	16.0
	RD	6.0	4.0	10.0

l – Moisture content and pH of soil in RC, RM and RD regions

No considerable variation of the moisture content in different months was observed. It was generally highest in distal region. The pH of the soil samples from three regions exhibited a narrow variation. In RC and RM it was always slightly alkaline whereas in the RD region it varied between 6.9 to 7.1 (Table 8).

Table 8
pH and moisture content /%/ of soil from crown /RC/, middle /RM/ and distal /RD/ regions of P. typhoides

Sampling months 1972	pH			Moisture content		
	RC	RM	RD	RC	RM	RD
January	7.1	7.2	6.9	14.5	15.0	17.0
February	7.1	7.3	6.9	14.5	16.0	15.6
March	7.4	7.3	6.9	13.5	14.3	14.5
April	7.4	7.4	6.9	14.6	15.7	15.2
May	7.5	7.4	6.9	15.6	16.2	16.2
June	7.5	7.3	7.1	16.5	15.6	17.6

DISCUSSION

During the course of present investigation, it was observed that the roots of *P. typhoides* decomposing in natural field conditions, in sterilized soil separately inoculated with certain fungal isolates and in the field soil maintained at different moisture levels, produced vanillic acid 3 - 4 dihydroxy benzoic acid and hydrocinnamic acid (Figs. 1 - 3) which proved toxic to six succeeding crop seeds and seedlings (Figs. 6 - 8) and certain soil fungi (Tables 4 - 6).

The production of phytotoxic substances by decomposing plant residues has been reported by many workers (Bonner 1950; Loehwing 1937; Kanaujia 1973; Patrick 1955, 1971; Patrick, Koch 1958, 1963; Patrick, Toussoun 1965; Patrick et al. 1963, 1964; Ripley 1941).

In normal field practice, the roots along with varying amounts of above and under ground plant parts of the crop are left in the field after the harvest. The field containing plant residued in different amounts is ploughed and sown by the succeeding crops. The field at this stage contains enough moisture which helps in the germination of the seeds. Simultaneously, the soil conditions also help in the decomposition of plant residuesby the activity of various microbes. The release of phytotoxins during decomposition of plant remains under such conditions has been reported and this was observed in the present study too. The active degradation of celluloses, hemicelluloses and lignins occurred during March and April (Table 7.). The conditions of the field along with a specific set of microorganisms engaged in the decomposition of roots possibly led to the production of certain intermediate breakdown products toxic to plants (Miller 1955; Miller et al. 1958; Patrick, Toussoun 1965). During March, the moisture content of middle and distal regions was comparatively higher than that of the crown region (Table 8). Higher moisture and low temperature of the soil are known to increase the toxic production (Kanaujia 1973; Patrick 1955; Patrick, Koch 1963). In April, sufficient degradation of roots had been observed in the crown region also. Light

showers in the month of April increased the moisture content and decreased the temperature which possibly led to the production of toxins in this region also (Fig. 1).

In one experiment where 15 dominant root-surface fungi were separately inoculated in sterilized soil, it was observed that toxin production observed only in six sets (Fig. 2). The different behaviour of microorganisms in soil and in pure culture is not fully known. Competition among the microorganisms for space and nutrition and colonization finally resulted in the varying decomposition abilities of the organisms (C o c h r a n e 1948; G a r r e t t 1963). The decomposition pattern is possibly so changed that various intermediate compounds formed during decomposition are not the same in each case and this resulted in the detection of two dissimilar phytotoxins in the case of the set inoculated with *Paecilomyces fusisporus*. Common plant residues, nearly edaphic conditions and probably parallel decomposition in these sets acounted for the production of vanillic acid in all of them (Fig. 2).

It was noticed that roots decomposing at 50 and 60% moisture levels produced phytotoxins earlier than comparatively drier and more moist soils. The breakdown products of celluloses and other constituents of the roots decomposed at various moisture levels at a particular time which in turn brings about variety of changes in the soil system. This could be argued that the activities of the microorganisms at 50 and 60% sets were such as to produce toxins more efficiently. The decomposition of the roots at low moisture levels for a long duration also led to the production of toxins which may possibly be due to the changed decomposition pattern by the same set of microorganisms.

The waschings of the decomposing roots in the present study variously lowered the germination of the six crops (Fig. 6 - 7) and reduced the seedling growth (Fig. 8), germination of certain fungal isolates was also lowered by the root-washings (Tables 4 - 6). Moreover, the decreased fungal population of the sets where toxins were liberated (Fig. 1 - 5 and Table 1) also confirms the toxic nature of the root-washings. Such substances from the decomposing roots seem to play an important role in the succession of fungi on roots and in the emergence of the crops.

As pointed out by D o r e n (1928), M i l l e r (1955), P a t r i c k and T o u s s o u n (1965), R i p l e y (1941) and S k i n n e r (1918), the soil toxins due to the organic components are mainly associated with heavy soils characterized by poor aeration, excessive moisture and relatively cool temperature conditions. Under oxygen-defficient conditions, proteins, celluloses, lignin and other constituents of plant residues decomposing in the soil produced a variety of intermediate compounds, many of which are phytotoxins. The above findings have been supported by P a t r i c k (1955, 1971) and P a t r i c k and K o c h (1958). The accumulated knowledge of phytotoxin production by

decomposing plant remains in the soil is mainly based on green-house and laboratory experiments (Patrick 1955, 1971; Patrick, Toussoun 1965; Snyder et al. 1959, Toussoun et al. 1968). Their production in field conditions is less known and this has been successfully carried out by the author.

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