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Influence of thiobencarb and fluchloralin on the soil and rhizosphere microflora of potato

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[m]Spray application at locally recommended rates of the herbicides thiobencarb and fluchloralin to potatoes reduced the rhizosphere and general soil microbial populations. Within 30 days the populations had generally recovered although there were some changes in the species composition of the fungal community that persisted until the final sample at harvest time.

Introduction

Foliar application of herbicides may alter the population and activity of microorganisms in the soil (Wainwright, 1978) which, in turn, can effect soil fertility. However, most research on this subject has been on a laboratory scale using non-rhizosphere soils (Chen et al., 1981; Roslycky, 1982; Tu, 1981) and there is but little information from field trials (Korpraditskul et al., 1989). Further, although the rhizosphere is one of the most important sites of microbial activity in soil, the effects of herbicides on the rhizosphere microflora has received little attention since most studies have dealt with soil microorganisms in general. There have, however, been some relevant studies on rhizospheres using, for example, those of corn (Smirnova, 1963), wheat (Catska & Loos, 1984), but there is no information on the effect of herbicides on the rhizosphere microflora of potato (*Solanum tuberosum* L.). This study was undertaken to help fill the last gap by investigating the effects of foliar application of some herbicides commonly used in India on the rhizosphere microflora of potato.

Materials and methods

Disease free tubers (cv. Kufri Jyoti), obtained from the Central Potato Research Station (CPRS) Shillong, were planted in the last week of March 1986 on farms of the CPRS, Shillong (altitude 1706 m, latitude 25°34′N, longitude 91°56′E). There were 38 plots of 1 m² for each treatment and for the control, all set out in a fully randomized design. The herbicides were those recommended for and commonly used locally on potato. The chemical names, doses used, and manufacturers of the herbicides were as follows: thiobencarb at 1500 g a.i. in 400 l water/ha (Saturn EC, 50% a.i. Pesticides India, Udaipur) and fluchloralin at 300 g a.i. in 100 l water/ha (Basalin EC 48% a.i., BASF India Ltd., Bombay). Pretreatment soil samples were collected and the herbicides sprayed 20 days after plant emergence; further samples were collected 0, 15, 30, 45 and 60 days after spraying.

To obtain the rhizosphere samples, the complete root system was dug out with a sterilized trowel and put into a sterilized polythene bag where it was tapped gently to remove loosely attached soil. Approximately 5 g of the roots were transferred to a 500 ml conical flask containing 100 ml sterile distilled water and a suspension of the rhizosphere soil was prepared by shaking the flask by hand for 10 minutes. Serial

dilutions were prepared from this suspension by initially transferring 10 ml of the suspension to 90 ml of sterile distilled water. Corresponding non-rhizosphere soil samples were collected at each sampling time from the depth in the soil corresponding to the root zone and sub-samples of 10 g were added to 100 ml sterile distilled water and used to prepare the initial non-rhizosphere suspensions. From each suspension, 0.5 ml of the appropriate dilution (1:1000 and 1:10000 for the fungal and bacterial populations respectively) was inoculated onto sterilized Petriplates containing 20 ml of rose bengal agar medium (Martin, 1950) for the enumeration of fungi or onto nutrient agar medium (Johnson & Curl, 1972) for bacteria. A specified volume of each of the suspensions used was dried at 105 °C for 24 hr in a hot air oven, cooled to room temperature and then weighed to determine the dry weight of soil per unit volume of the suspension.

Petriplates for fungi were incubated at 25 °C for seven days and for bacteria at 30 °C for 24 h. Populations were calculated per gram dry weight of soil and three replicates were used for each estimate.

Fungi were identified from keys provided by Barnett & Hunter (1972), Subramanian (1971) and Domsch et al. (1980).

Results and discussion

Both herbicides affected the microbial populations of both rhizosphere and non-rhizosphere soil. The fungal population in the soil from treated plants initially decreased and then increased for up to 45 days after which it again decreased as it did in soil from the control plots (Fig. 1a). Thiobencarb treatment reduced the fungal population more than did treatment with fluchloralin. The initial population decreases may have been caused by fungitoxicity of the herbicides and it would be helpful to examine this hypothesis by using selected fungal isolates for in vitro tests with each herbicide separately, using the active ingredient alone, not the commercial formulations. Recovery was probably due to dilution, leaching and physiochemical or biological breakdown of the herbicides. The stimulation of non-rhizosphere fungal numbers in the fluchloralin treated plots seems contrary to the above hypothesis but an explanation could be sought by examining individual isolates for different responses. For example the results obtained by Olsen et al. (1984) with a different crop, soil and climate showed that trifluoralin had no effect on populations in either general soil or in the rhizosphere. Zelles et al. (1985) noted that most herbicides at low doses stimulate the microbial activity but at high doses they are generally inhibitory.

The fungal species isolated from the rhizosphere and non rhizosphere soils of treated and control plants are given in Tables 1 and 2. *Arthrobotrys arthrobotryoides, Fusarium moniliforme, Gonytrichum macrocladum, Humicola fuscoatra, Nectria ventricosa* and *Periconia macrospinosa* were isolated only from herbicide treated plants. *Fusarium poae, F. moniliforme, Gonytrichum macrocladum, Oidiodendron echinulatum* and *Penicillium frequentans* were isolated only from rhizosphere soil while *Arthrobotrys conoides, A. oligospora, A. arthrobotryoides, Humicola fuscoatra, Mortierella minutissima,* and *Nectria ventricosa* were isolated from non-rhizosphere soils. *Cladosporium cladosporioides, Fusarium oxysporum, F. solani, Mucor hiemalis, M. plumbeus, M. racemosus, Penicillium brevicompactum, P. chrysogenum, Trichoderma harzianum, T. viride* and sterile forms were common to all the treated as well as untreated soils.

Several fungi, for example *C. cladosporioides, F. oxysporum, Mucor* spp., *Penicillium* spp. and *Trichoderma* spp., were found throughout the study, suggesting that they are tolerant to the two herbicides. Cullimore (1971) also reported that *Fusarium oxysporum, Trichoderma lignorum* and *Penicillium* spp. were resistant to herbicides. Species of *Penicillium* and of *Trichoderma* are also able to degrade herbicides (Kaufman, 1966).

Variation in the bacterial counts (Fig. 1b) followed a pattern generally similar to that of the fungi. Thiobencarb depressed bacterial numbers proportionally less than it did those of fungi. The bacterial population in the control soils remained always higher than in the herbicide treated soils.

Microbial populations may be stimulated (Chen et al., 1981) or may fall and then recover as we found and as reported by Korpraditskul et al. (1989) and by Bopaiah & Rai (1979) for bacteria. Our results show that rhizosphere and non-rhizosphere populations were initially depressed by the application of herbicides but although they then recovered they still remained lower than in the control soils at the last sampling date at harvest time.

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HERBICIDE EFFECTS ON SOIL AND RHIZOSPHERE MICROFLORA

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Fig. 1. Effect of herbicides on bacterial and fungal populations in rhizosphere and non-rhizosphere soil (× __ × control; ○ __ ○ thiobencarb; □ __ □ fluchloralin).

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Table 1. Effect of herbicides on the fungal population ($\times 10^4$, g^{-1} dry soil) of rhizosphere soils of potato.

Isolates	Control					Benthiocarb				Fluchloralin			
	0	15	30	45	60	15	30	45	60	15	30	45	60
Cladosporium cladosporioides[a]	11.7	20.0	14.8	56.1	45.2	49.2	22.2	25.1	15.6	18.9	73.0	74.0	10.5
Fusarium moniliforme	–	–	–	–	–	5.8	6.0	6.0	12.2	–	–	–	–
Fusarium oxysporum	–	–	2.0	7.0	6.8	–	8.0	20.1	4.0	–	7.0	6.0	5.0
Fusarium poae	–	–	8.0	11.3	9.2	–	–	–	–	–	–	–	–
Fusarium solani	–	–	–	8.2	4.0	–	–	–	–	–	5.8	9.2	3.0
Gonytrichum macrocladum	–	–	–	–	–	–	–	–	–	–	–	14.1	6.0
Monilia	2.0	4.1	4.0	–	–	–	–	–	–	–	–	–	–
Mucor hiemalis	9.7	19.0	58.0	85.0	12.2	8.8	20.2	17.6	15.4	–	15.2	48.0	20.8
Mucor plumbeus	12.5	25.3	36.2	18.5	10.3	5.0	10.3	21.8	18.9	–	19.7	44.9	23.0
Mucor racemosus	2.5	9.0	17.0	19.2	15.9	–	18.0	21.7	12.3	8.0	12.6	17.7	9.3
Oidiodendron echinulatum	–	–	–	–	–	–	–	12.1	–	–	–	–	–
Penicillium brevicompactum	5.0	25.7	23.8	28.4	21.3	–	–	–	–	–	–	–	–
Pencillium chrysogenum	10.8	17.0	10.6	27.2	22.8	–	–	8.2	4.0	5.9	17.1	14.8	10.2
Penicillium frequentans	–	–	–	–	–	–	13.4	–	–	–	18.0	29.6	20.4
Trichoderma harzianum	–	–	–	–	–	–	3.0	3.4	2.4	4.5	4.9	6.2	–
Trichoderma viride	–	–	–	5.6	4.4	–	–	5.8	3.0	–	–	–	–
Sterile white	–	–	5.0	6.3	5.0	–	–	7.8	1.0	–	6.3	11.1	2.5
Sterile orange	–	–	–	–	–	–	–	–	–	–	–	55.8	3.0

[a] See Table 3.

Table 2. Effect of herbicides on the fungal population ($\times 10^3$, g^{-1} dry soil) of rhizosphere soils of potato.

Isolates	Control					Benthiocarb				Fluchloralin			
	0	15	30	45	60	15	30	45	60	15	30	45	60
Arthrobotrys arthrobotryoides[a]	–	–	–	–	–	–	3.7	1.5	1.8	–	–	–	–
Arthrobotrys conoides	1.1	1.5	1.5	1.5	1.7	–	–	–	–	–	–	–	–
Arthrobotrys oligospora	1.1	1.5	1.5	1.7	1.5	–	–	–	–	–	–	–	–
Cladosporium cladosporioides	–	–	2.2	8.9	2.0	–	14.9	25.2	22.5	21.6	30.8	24.7	30.9
Fusarium oxysporum	–	–	8.8	17.8	–	–	7.6	8.0	8.4	1.5	1.5	3.0	–
Fusarium solani	–	–	–	9.3	9.3	–	–	–	–	–	–	6.0	–
Humicola fusco-atra	–	–	–	–	–	–	2.3	5.1	1.4	–	–	–	–
Monilia	–	–	–	4.7	6.9	–	–	–	–	–	–	–	–
Mortierella minutissima	–	–	–	4.2	2.0	–	–	–	–	–	–	–	–
Mucor hiemalis	8.0	8.0	1.3	–	–	2.7	4.4	6.1	2.8	8.2	–	8.8	9.1
Mucor plumbeus	7.0	9.4	7.1	5.6	5.0	–	–	–	–	–	–	5.9	–
Mucor racemosus	8.0	8.6	4.2	4.2	–	2.7	4.9	2.6	1.8	–	–	–	–
Nectria ventricosa	–	–	–	–	–	–	–	–	–	–	3.2	2.7	–
Penicillium brevicompactum	4.0	4.0	6.0	4.0	4.0	–	2.3	2.7	3.8	3.1	5.1	6.9	4.9
Penicillium chrysogenum	3.0	3.8	6.7	6.2	7.7	5.0	6.3	8.7	8.0	–	3.2	2.9	–
Periconia macrospinosa	–	–	–	–	–	–	–	–	–	–	–	–	8.8
Trichoderma harzianum	–	–	2.0	4.2	–	–	1.6	5.2	2.0	–	8.7	2.7	6.6
Trichoderma viride	–	–	–	4.7	7.9	–	–	6.8	2.1	–	–	–	–
Sterile White	7.0	5.9	3.8	6.1	–	5.8	3.5	3.9	8.6	5.9	5.7	4.8	7.2
Sterile Orange	9.0	9.7	10.9	14.2	16.3	4.1	3.7	6.0	9.4	3.9	5.2	7.8	6.0

[a] See Table 3.

Table 3. Authorities for the binomials cited in Tables 1 and 2.

Arthrobotrys arthrobotryoides (Beal) Lindau	*Mucor hiemalis* Wehmer
A. conoides Drechsler	*M. plumbeus* Bon
A. oligospora Fres	*M. racemosus* Fres
Cladosporium cladosporioides (Fres) de Vries	*Nectria ventricosa* Booth
Fusarium moniliforme Sheld	*Oidiodendrum echinulatum* Barrow
F. oxysporum Schlecht emend Snyder et Hans	*Penicillium brevicompactum* Dierckx
F. poae (Peck) Wollenw	*P. chrysogenum* Thom
F. solani (Mart) Appel en Wolenw	*P. frequentans* Westling
Gonytrichum macrocladum (Sacc) Hughes	*Periconia macrospina* Lefebvre et Johnson
Humicola fusco-atra Traaen	*Trichoderma harzianum* Rifai
Mortierella minutissima Van Tiegh	*T. viride* (Pers ex Fr) Gray