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Allelopathic effect of orchard soils on seedling growth of rough lemon (*Citrus jambhiri* Lush.)

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Abstract

An experiment was conducted to study the allelopathic potential of old orchard soils on the seedling growth of rough lemon. Soils from the root zone spheres of eight orchards of mango, aonla, peach, pomegranate, citrus, pear, ber, guava and virgin soil as a control was used for raising the seedlings. The rough lemon *Jatti khatti* seedlings of one and a half year old raised in aonla, ber and peach orchard soil as growing media showed the reduction in shoot length (40-50%), leaf number (46-63%), leaf area/ plant (62-69%) and shoot dry weight (79-83%). The root length was most inhibited by ber, aonla and peach orchard soils. The percent reduction in root dry weight (11.23- 34.48%) of the seedling was not in equal proportion to reduction in root volume (42.55- 55.86%). Root dry weight density varied between 0.55-0.96 g mL^{-1} and root: shoot ratio between 1.42-1.82. Whereas, in citrus, mango, pomegranate, ber and guava orchard soils, the percent reduction in root dry weight was in equal proportion to root volume and root dry weight density varied between 0.41-0.49 g mL^{-1} and root: shoot ratio between 0.44-0.72. The shoot and root growth of the seedlings was at par when raised in citrus and pomegranate orchard soil as growing media. Leaf N and P contents increased, whereas, Ca and Mg decreased in all the orchard soils except citrus and pomegranate orchard soils as growing media. Leaf Fe, Cu and Mn contents in all the orchard soils as growing media were in toxic range except citrus and pomegranate orchard soils. Overall, the orchard soils of deciduous fruit plants showed more allelopathic effect than the soils growing evergreen fruit plants in citrus cultivation.

Key words: Allelopathy, *Citrus jambhiri*, old orchard soils, seedling growth, nutrients.

Introduction

Allelopathy is an important mechanism of plant interference and is mediated through the addition of chemicals to the plant environment. However, the difficulty in replanting fruit trees following the removal of old orchard has been recognized for many years in U.S.A. and Europe. Most of the research indicated that numerous allelopathic compounds are released into the environment by root exudations, leaching, decomposition and volatilization (Rice, 1984). In monoculture of annuals and cultivation of perennial plants in orchards and grasslands where the same substrate is used many times, there is more build up of homogenous metabolites and plant residues in the soil. Besides, living plants exuding the allelochemicals, microbial decay of plant residue also release the toxic metabolites into the soil (Politycka, 2005). They are present in the roots, rhizomes, stems, leaves, flowers, pollens, fruits and seeds but the leaves are the major source. Their concentration varies with age, season, plant parts and growth habit etc. (Narwal, 1994). The phytotoxic potential of these chemicals depends on plant type, activity of microflora, their release and removal from the soil solution or immobilized by the plant uptake, adsorption to the soil particles and degradation by micro-organism (Kobayashi, 2004). Allelochemicals impair the physiological function of the cell directly or indirectly, thereby retard plant growth and cause soil sickness in various fruit crops (Hassan *et al*., 1989 and Arora *et al*., 2002 in citrus; Ercisli and Turkkal, 2005 in walnut; John *et al*., 2007 in pomegranate; Sharma *et al*., 2000 in mango; Oudhia, 2001 in guava; Alshahrani *et al*., 2009 and Arya *et al*., 2011 in ber and aonla). However, most of the research has been conducted on fruit trees-field crops and weeds–fruit trees interactions, studies on allelopathy aspects of interaction among fruit tree species is still lacking. Understanding of interaction will help in solving the problem in success of replanting. Keeping in view the above facts, the present investigation was carried out to find out the allelopathic effect of different old orchard soils on the seedling growth of rough lemon syn. *Jatti khatti* Lush.

Materials and methods

The experiment was carried out at PAU, Regional Station, Bathinda (Punjab) during 2008 and 2009. Bulk soils were collected from virgin soil and root zone sphere (0-40 cm depth and 30-60 cm radial distances from the trunk) of three plants, each aged 15-19 years from mango, aonla, peach, pomegranate, ber, pear and guava orchard during 2nd fortnight of February. The soil collected from each treatment was mixed thoroughly and analyzed for pH, EC, OC, phosphorus and potassium content. pH ranged from 8.00-8.21; EC - 0.15-0.23 dsm^{-1}; OC- 0.32-0.45%, P - 11-17 kg ha^{-1} and K- 161-256 kg ha^{-1}. The roots, which were collected from root zone sphere site, were chopped, dried and these chopped root bits were mixed with the soil @ 60g/pot. The cemented pots of 40 cm diameter and 40 cm depth capable of holding 25 kg air dried soil were filled with soil and saturated with the water before sowing of the seeds. The freshly extracted seeds of rough lemon (*Citrus jambhiri* Lush.) collected from single tree were sown during 1st week of March and the pots were placed in open nursery area. Three pots were maintained per treatment and after germination, 30 seedlings were maintained/ pot. The seedling irrigated as and when required with equal amount of canal water to prevent leaching from the bottom of the pots. Weeds were removed manually and insects were controlled chemically. These seedlings were allowed to grow for about one and half year. The seedlings were uprooted, washed and separated into shoots and roots. The length of shoots and roots were measured with meter scale and shoot diameter at 2-inch height with Vernier Caliper. Root volume was determined with water displacement method and leaf area was measured with leaf area meter. Dry weight was observed after drying in an oven at 60 ^{0}C till the constant weight was noticed. Root dry weight density was calculated by the ratio of root dry weight to root volume and expressed as g/mL. Leaf samples were collected during September, washed, oven dried, ground and digested with diacid (H_2SO_4: $HClO_4$) in the ratio of 4:1 for macronutrients contents, and with (HNO_3: $HClO_4$) for micronutrients. Nitrogen was estimated with Nesseler's reagent method and phosphorus by Vando Molybdo Yellow colour method, potassium with flame photometer and Ca and Mg colorimetricaly. Micronutrients were estimated with Atomic Absorption Spectrophotometer. Trials were carried out in a completely randomized design with three replications. Data were subjected to ANOVA and means were compared by Duncan's multiple range test.

Results and discussion

Shoot growth: The shoot growth of the rough lemon rootstock seedlings in terms of shoot length, stem diameter, leaf area, shoot dry weight and total dry weight was significantly inhibited by old orchard soils (Table 1). The maximum reduction in shoot length, stem diameter, number of leaves was observed in seedlings raised in aonla orchard soil followed by ber orchard soils. Whereas, the maximum reduction in leaf area and total dry weight of the seedlings was found in ber orchard soil followed by aonla grown orchard soil. The minimum shoot dry weight of seedlings was observed in peach orchard soil which was at par with aonla and ber grown soil. Shoot dry weight and total dry weight of the seedlings raised in mango, pear and guava orchard soils was found at par with each other. The citrus orchard soil also exhibited significantly inhibitory effect on the growth of rough lemon seedling *i.e.*, shoot length, stem diameter, leaf count, leaf area, shoot dry weight and total weight which were at par with pomegranate orchard soil. Rough lemon seedlings raised in aonla, ber and peach orchard soil recorded the maximum reduction in shoot length (≈40-50%), leaf number (≈46-63%) and leaf area/ plant (≈62-69%) and shoot dry weight/ plant (≈79 - 83%). Rough lemon seedlings behaved differently in different orchard soils. This may be due to differential response of rough lemon rootstock to different old orchard soils as it is very sensitive to allelo-chemicals (Singh and Achhireddy, 1987) and secondly, the quantitative as well as qualitative differences in the accumulation of allelochemicals by different fruit plants and their phytotoxic potential. These results are in conformity with those of Narwal (1994) that concentration of allelochemicals varies with season, plant parts and growth habit and phytotoxic potential of these chemicals depends upon plant type, their release and removal from the soil particles (Kobayashi, 2004). Seedlings raised in citrus and pomegranate orchard soil behaved similarly in all the growth aspects as there was same proportion of decrease in shoot and root growth and biomass production as compared to control. Saroj *et al.* (2002) reported that ber leaf extract had variable response on mustard, cluster bean and wheat vigour index. Jadhav (2003) found that leaf extract of aonla inhibited the growth of the field crop. Arya *et al.* (2011) reported the inhibited growth of mustard, moth bean, cluster bean and brinjal with the leaf extract of ber and aonla. Similarly, Alshahrani *et al.* (2009) reported that leaf extract of *Zizyphus Spina*- Christi was inhibitory to the growth of *Prosopsis juliflora*.

Root growth: Root growth of the seedlings was found significantly altered when raised on various old orchard soils (Table 2). The minimum primary root length was measured in seedlings raised in peach orchard soil which was statistically at par with ber, aonla and pear orchard soil. The maximum root length of seedlings was measured in control which was at par with citrus, mango, pomegranate and guava orchard soil. Root volume was least in ber orchard soil followed by peach orchard soil whereas it was highest in control. Similar results were earlier reported by Arora *et al.* (2009) that maximum primary root density was observed in mango and guava and minimum in ber orchard soils irrespective of citrus rootstocks. Root dry weight of the seedlings raised in virgin soil (control) was at par with peach orchard soil. The least root dry weight was measured in ber orchard soil which was at par with guava orchard soil. The seedlings raised in citrus and pomegranate orchard soil attained almost similar root dry weight 4.07 and 4.13g/ plant, respectively and in mango and aonla orchard soil 4.93 & 4.87g/ plant, respectively. Whereas, in peach, aonla and pear orchard soil, the root dry weight decreased from 11.23-34.48% and root volume from 42.55-55.86%. Hence root dry weight density varies from 0.55-0.96g/mL and root: shoot

Table 1. Effect of different old orchard soils on the shoot growth and dry weight of Rough lemon rootstock seedlings

Treatments (Growing media)	Shoot length (cm)	Stem diam. (cm)	Leaf count/ plant (Nos.)	Leaf area/ plant (cm^2)	Leaf area/ leaf (cm^2)	Shoot dry wt./ plant (g)	Total dry wt. (g)
Virgin soil (control)	68.60^f	0.84^e	106.67^e	877.21^f	8.22^e	18.99^d	25.38^f
Citrus orchard soil	55.35^e	0.65^d	85.67^d	664.15^e	7.75^d	9.21^c	13.27^e
Mango orchard soil	37.45^{ab}	0.59^{cd}	87.00^d	533.25^d	6.13^b	6.77^b	11.70^{de}
Aonla orchard soil	34.01^a	0.52^a	39.50^a	287.51^{ab}	7.28^c	3.42^a	8.29^{ab}
Peach orchard soil	41.76^c	0.54^a	57.33^{bc}	330.80^{bc}	5.77^{ab}	3.13^a	8.82^{bc}
Pear orchard soil	44.97^d	0.58^{bc}	66.00^c	376.98^c	5.71^a	6.88^b	11.09^{cde}
Ber orchard soil	37.72^{ab}	0.52^a	48.50^{ab}	267.56^a	5.52^a	3.95^a	6.47^a
Pomegranate orchard soil	56.81^e	0.66^d	84.67^d	627.68^e	7.14^{cd}	8.89^c	13.03^e
Guava orchard soil	46.92^d	0.62^{cd}	92.33^d	564.69^d	6.11^b	6.47^b	9.82^{bcd}

Mean (n=12) separated by Duncan's Multiple Range Test. Values followed by different letter within a column are significantly different (P<0.05) from other values in the same column.

ratio between 1.42-1.82 which may be either due to change in root morphology/ architecture and accumulation of allelochemicals and mobilization of more metabolites from shoot to roots under stress caused by allelochemicals produced by these plants or both. Hence, it seems that these plants produced chemicals which accumulates in roots and causes stress to seedlings hence increased weight proportionally. A shift in relative allocation of biomass to roots has been earlier observed under condition of environmental stress (Alshahrani *et al.*, 2009; Rutherford and Powrie, 1993). In citrus, mango, pomegranate and guava, the percent reduction in root dry weight was in equal proportion to root volume; hence root dry weight density varied between 0.41 to 0.49g/ mL. This may be due to less difference in partitioning of metabolites in seedling raised in these orchard soils. Hassan *et al.* (1989) reported that allelopathy is at least partly involved in the citrus replant problem. John *et al.* (2007) reported that the nature and degree of allelopathic effects of trees varied with crop species. Aonla, peach, pear are the winter deciduous and shed their leaves in winter months and due to slow decomposition under low temperature in these months it may lead to accumulation of toxins in the soil. Ber is the summer deciduous which sheds its leaves in month of May followed by heavy monsoon rain in July, so there in very fast decomposition of leaves due to high temperature and rainfall and hence more accumulation of toxins in the soils. Earlier, Narwal (1994) reported that leaves are the major source of allelochemicals and concentration varies with season and growth habit. Citrus, mango, pomegranate and guava plants are evergreen and there is very less litter fall and hence there is less production of toxins which may either be degraded or leached out with the passage of time so there is less inhibitory effect by these plants. The accumulation of allelochemicals in soil to the toxic level is decided with difference between the speed of allelochemicals release and their degradation (Wojcik – Wojtkowiak *et al.*,1998). These results are in conformity with those of Arya *et al.* (2011), Oudhia (2001) and Sharma *et al.* (2000) in field crops with mango, guava, ber and aonla leaf leachates.

Leaf macronutrients: Leaf macronutrients contents *i.e.*, N, P, K, Ca and Mg differed significantly among the various treatments (Table 3). The maximum N content in the seedlings of rough lemon was estimated when grown in guava orchard soil which was at par with mango and pear orchard soil and it was minimum in citrus and pomegranate orchard soil, which was at par with control. Singh and Achhireddy (1987) found that rough lemon grown with *Lantana* had higher N content than when grown alone. Seeding raised in pomegranate orchard soil contained P which was at par with control and citrus orchard soil. Maximum P accumulation was in the seedling of rough lemon raised in ber and peach orchard soil followed by guava (0.20%) and aonla orchard soil. Potassium content varied between 1.75 to 1.92 %. The maximum value was observed in ber and minimum in guava orchard soil. Buchholtz (1971) reported reduced N, P and K uptake by corn due to allelopathic effects of *quack grass*. He further stated that the inhibition or stimulation of N, P and K uptake in both corn (*Zea mays*) and soybeans (*Glycine max* L.) by several weed residue was not consistent, and relationship between inhibitory effects and nutrients uptake were not found. Calcium content was found maximum in citrus orchard soil raised seedling closely followed by control. The minimum leaf Ca content was observed in ber orchard soil, whereas in pear, peach, aonla and guava orchard soil the rough lemon seedlings leaf Ca content

Table 2. Effect of different old orchard soils on the root growth, root dry weight and root: shoot ratio of Rough lemon rootstock seedlings

Treatments (Growing media)	Primary root length (cm)	Root volume (mL)	Root dry wt. (g)	Root dry wt. density (g/ mL)	Root: Shoot dry wt. Ratio
Virgin soil (control)	39.61[c]	13.16[f]	6.41[e]	0.487[a]	0.337[a]
Citrus orchard soil	39.41[c]	8.44[d]	4.07[bc]	0.482[a]	0.442[b]
Mango orchard soil	38.37[c]	10.52[e]	4.93[cd]	0.469[a]	0.728[d]
Aonla orchard soil	39.61[c]	6.42[bc]	4.87[cd]	0.758[c]	1.424[e]
Peach orchard soil	32.52[a]	5.94[b]	5.69[de]	0.958[d]	1.818[f]
Pear orchard soil	34.76[ab]	7.56[cd]	4.20[bc]	0.555[ab]	0.610[c]
Ber orchard soil	33.64[a]	4.28[a]	2.51[a]	0.586[b]	0.635[cd]
Pomegranate orchard soil	37.17[bc]	10.57[e]	4.13[bc]	0.400[a]	0.464[b]
Guava orchard soil	37.82[bc]	6.82[c]	3.34[ab]	0.490[a]	0.516[bc]

Mean (n=12) separated by Duncan's Multiple Range Test. Values followed by different letter within a column are significantly different (P<0.05) from other values in the same column

Table 3. Effect of different old orchard soils on the leaf nutritional status of Rough lemon rootstock seedlings

Treatments (Growing media)	Macronutrients (%)					Micronutrients (ppm)			
	N	P	K	Ca	Mg	Zn	Fe	Cu	Mn
Virgin soil (control)	1.66	0.12	1.82	3.54	0.70	15	62	98	16
Citrus orchard soil	1.47	0.12	1.77	3.62	0.60	15	65	93	19
Mango orchard soil	2.33	0.14	1.72	3.22	0.58	16	71	104	15
Aonla orchard soil	2.14	0.18	1.88	3.15	0.54	14	79	134	21
Peach orchard soil	1.90	0.22	1.77	3.10	0.54	14	75	174	27
Pear orchard soil	2.24	0.16	1.77	3.10	0.48	13	81	142	21
Ber orchard soil	2.08	0.22	1.92	2.80	0.38	13	109	124	32
Pomegranate orchard soil	1.47	0.10	1.82	3.45	0.62	18	62	90	19
Guava orchard soil	2.39	0.20	1.75	3.27	0.52	12	62	104	20
C D (5%)	0.21	0.06	NS	0.14	0.11	1.4	12	18	3.0

ranged from 3.10-3.27%. The maximum Mg content was found in control which was statistically at par with citrus and pomegranate orchard soil raised seedling. The minimum leaf Mg content was observed in ber orchard soil. The leaf Mg content ranged from 0.48- 0.54% in pear, aonla and peach orchard soil. Ca and Mg uptake was inhibited by all the treatments except citrus and pomegranate orchard soils and the inhibition was more where there was more growth inhibition. It was observed that all orchard soils as growing media played an important role in decreasing the capacity for cation uptake except citrus and pomegranate and has a positive effect on uptake of plant nutrients such as N, P, Fe, Cu and Mn. Similar results were earlier reported by Ercisli and Turkkal (2005).

Leaf micronutrients: The maximum leaf Zn content in the seedlings of rough lemon was observed in pomegranate orchard soil which was significantly better than the other treatments and minimum was recorded in guava orchard soil. Maximum leaf Fe content was in seedlings grown in ber orchard soil, whereas in other treatments it ranged from 62-79 ppm only. The maximum Cu content was found in seedlings raised in peach and minimum in pomegranate orchard soils. In citrus, mango and guava orchard soil, the leaf Cu content varied from 93-104 ppm, whereas, it varied from 124-142 ppm in ber, aonla and pear orchard soil raised seedlings. Manganese content of leaves was found maximum in ber orchard soils which was at par with peach orchard soil and was minimum in mango orchard soil grown seedlings. Overall, Fe, Cu, and Mn contents were higher than reported in literature which may have caused toxicity to the rough lemon seedlings and hence reduced the growth. Thus, our study shows that where growth was inhibited more there was more nutritional imbalance such as in ber, peach, aonla, pear, guava and mango orchard soils. The decrease in nutritional status in citrus and pomegranate orchard soils was less as compared to other growing media. This may be either due to dilution factor or high uptake. Thus, it is reasonable to assume both direct and indirect effects of allelochemicals on plant water relationship and accumulation of stress producing chemicals, which in turn cause reduction in plant growth and translocation of metabolites and imbalance/ inhibition/ stimulation of nutrients.

It may be concluded that citrus rootstock tested showed different and relatively consistent pattern of sensitivity to old orchard soil. So, it can be inferred that allelochemicals may be selective in their action, or plants may be selective in their response. Allelochemicals produced by the different fruit plants affected the citrus rootstock growth in one or the other way by inhibiting root/ shoot growth or both. This study further indicates that deciduous fruit plants like ber, peach, aonla and pear have more allelopathic potential than evergreen fruit plants *viz*., citrus, mango and guava in citrus cultivation. This study may help to know the fruit tree to fruit tree interaction and to resolve the problem faced in reorcharding.

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