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Intercropping and weed management effect on soil microbial activities in newly planted mango and citrus orchards

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ABSTRACT

Application of herbicides and other agro-chemicals used in agriculture affects the vital functions and population dynamics of soil microorganisms. Soil microbial population was assessed in 30 days interval up to 90 days in mango and citrus orchards. Among the treatments, intercropping of greengram-pea-greengram recorded higher bacterial population (21.7 x 10^6cfu/g), was followed by intercropping of cowpea-pea-cowpea (19.5x10^6 cfu/g) at 90 days after spraying. It was found that the highest fungi population was recorded in intercropping of greengram-pea-greengram combined with herbicide application treatment (14.4 x 10^3 cfu/g). Similarly, higher actinomycetes population was observed in intercropping of greengram-pea-greengram treatment (8.2 x 10^3cfu/g) followed by intercropping of cowpea-pea-cowpea (7.3 x 10^3cfu/g) in mango orchard. In citrus field, highest bacterial population was observed in intercropping of greengram-pea-greengram treatment (21.3 x 10^6cfu/g).This was followed by intercropping of cowpea-pea-cowpea (18.4 x 10^6 cfu/g). The maximum fungi population (14.8 x 10^3cfu/g) was observed in cowpea-pea-cowpea treatment and maximum actinomycetes (8.4 x 10^3 cfu/g) population was recorded in intercropping of greengram-pea-greengram treatment. Basal respiration was significantly more in treatments of intercropping systems. Among the treatments, intercropping of greengram-pea-greengram treatment (193 mg/kg of CO_2-C) had more basal respiration rate during *Kharif* season in mango orchard. Similar trends were observed in citrus orchard.

Key words: Actinomycetes, Bacteria, Citrus, Fungi, Intercropping, Mango, Soil respiration, Weed management

Intercropping is considered to be one of the most significant cropping techniques in sustainable agriculture. Besides diversifying agricultural output, intercropping also improves nutrient status and the physical properties of the soil. Due to wide spacing and developing root patterns, during initial years (up to 8-10 years) of mango and citrus orchards establishment, large unutilized interspace can be exploited for growing inter and mixed crops successfully and adequate management of the orchard. This enables the farmers to raise extra income during the years when the main crop yields no or low returns. Some fertility restoring crops like legumes and leguminous cover crops should be included into the intercropping patterns. Legume intercropping supporte continuous recycling of plant residues and improved microbiological transformations of nutrients into an available form.

Herbicides form the principal component of weed management in crops and cropping systems. The continuous use of herbicides may lead to many problems like residual toxicity, health hazards and mammalian toxicity. Many herbicides are directly applied to the soil, and if applied by other methods eventually reach the soil either as runoff, drift or washed down through atmospheric precipitation (Cork and Krueger 1991). Herbicides and their degradation products generally get accumulated in the top soil to a depth of approximately 15 cm, the zone of maximum activity of soil flora and fauna, and may upset the equilibrium of soil microflora thereby influencing the future soil fertility and the general growth and development of crop plants (Schuster and Schroder 1990). Hence, a study was carried out to investigate the impact of intercropping and herbicidal applications on soil microflora in mango and citrus orchard.

MATERIALS AND METHODS

The experiments were conducted at DWR, Jabalpur for two consecutive seasons (*Kharif* and *Rabi*) during 2009-10. The treatment consisted of intercropping of cowpea-pea-cowpea; moong-pea-moong; the combination of both the intercropping systems with fluchloralin/ pendimethalin/ fluchloralin in each season; metribuzin 0.5 kg/ha; glyphosate 2.0

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kg/ha; two mechanical weeding in each season and weedy check in randomized block design with three replications.

Enumeration of microorganisms

The soil samples were collected from 0-15cm profile in all the plots at before spraying and at the time of harvest. The soils were soaked into 90 ml deionized water at the amount of 10 g, respectively. This mixed liquor was shaken for 10 min and kept still for 5 min. one ml of the supernatant of the mixed liquor was diluted to proper dilution twice and inoculated in the diluted water at the constant temperature of 30º C. All samples were performed in triplicate, and were used for enumeration microorganisms. The viable microbial counts were analyzed by the standard technique of serial dilution and pour plating. Enumeration of bacteria and fungi were carried out in soil extract agar medium (James 1958) and Rose Bengal Agar medium (Parkinson *et al.* 1971). The Kenknight's Agar medium (Wellingtonn and Toth 1963) is used for enumeration of actinomycetes. After allowing for development of discrete microbial colonies during incubations under suitable conditions, the colonies were counted and the number of viable bacteria, fungi and actinomycetes expressed as colony forming units (cfu) per gram dry weight of soil by taking into account the soil dilutions.

Soil respiration

Soil respiration was determined by incubating moist soil samples (50 g, 60% field capacity) in a air-tight jar with a beaker containing of 10 ml 0.5 M NaOH for 10 days. The evolved CO_2 was measured by titration of excess NaOH with 0.25N HCl after addition of $BaCl_2$ to precipitate CO_3^{-2} ions. The concentration of CO_2-carbon was expressed as mg CO_2-carbon/ kg/10 days (Jenkinson and Ladd 1981).

RESULTS AND DISUSSION

The total bacterial population was significantly more in all the intercropping treatments at 90 days after spraying. With progress of time, population was increased in all the treatments. The maximum bacteria population was found in intercropping of greengram-pea-greengram treatment (21.7 x 10^6 cfu/g) during *Kharif*, this was followed by cowpea-pea-cowpea treatment (19.5 x 10^6 cfu/g) in mango during *Kharif* season (Table 1). Similarly, in citrus field, highest bacterial population was observed in intercropping of greengram-pea-greengram treatment (21.3 x 10^6 cfu/g). This was followed by intercropping of cowpea-pea-cowpea treatment (18.4 x 10^6 cfu/g) during *Kharif* (Table 2). This may be due to the fact that in intercropping conditions, more soil organic matter is available which provides nutrients to microorganisms resulting in their proliferation. Initially, herbicide applied treatments (30 DAS) had significantly less bacterial count than weedy control, which recovered later on (Table 1 and 2). Balasubramanian and Sankaran (2001) also reported initial suppression of soil microflora by the herbicide application in different soils. The toxic effects of herbicides normally appear immediately after the application when their concentration in the soil is highest. Later on, microorganisms take part in degradation process and herbicide concentration and its toxic effect decreases (Radivojevic *et al.* 2004).

In our present study in mango field, fungi population was more in intercropping of greengram-pea-greengram combined with herbicide application treatment (14.4 x 10^3 cfu/g) during *Rabi* followed by intercropping of greengram-pea-greengram (13.5 x 10^3 cfu/g) during *Kharif* season (Table 1). In citrus, results revealed that the highest fungi population were recorded under intercropping of cowpea-pea-cowpea (14.8 x 10^3 cfu/g) during *Rabi* (Table 2). There was not much difference in the fungi population of different treatments. This may be due to fact that the fungi might have become tolerant to herbicide and would have utilized the herbicide as a nutrient source. In mango, higher actinomycetes population was observed in intercropping of greengram-pea-greengram (8.2 x 10^3 cfu/g) followed by intercropping of cowpea-pea-cowpea (7.3x10^3cfu/g) during *Rabi* (Table 1). Similarly, maximum actinomycetes (8.4 x 10^3 cfu/g) population in intercropping of greengram-pea-greengram treatment during *Rabi* (Table 2) in citrus field. The dynamic increase of the microorganisms in the rhizosphere of fruit crops intercropped with legume cultivation can be explained by the favorable quantitative and qualitative composition of organic compounds provided in the form of root exudates and crop residues (Lehmann *et al.* 2000). This fact is confirmed by earlier information from the previous investigators (Al Yahyai 2009, Abouziena 2010). Significantly higher microbial populations in intercropping treatments at all stages of observation might be due to healthy and conducive environment for the microorganisms as compared to the control plots.

Intercropping has significant effects on microbiological and chemical properties in the rhizosphere, which may contribute to the yield enhancement by intercropping. An unintended consequence of application of herbicides is that it

Table 1. Effect of intercropping and herbicidal treatments on soil microbes in mango orchard

Treatment	Soil microbial population (cfu/g soil)								
	Bacteria x10^6			Fungi x10^3			Actinomycetes x10^3		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Kharif									
T_1- Intercropping of cowpea *fb* pea *fb* cowpea	7.5 (0.87)	18.2 (1.26)	19.5 (1.29)	4.2 (0.62)	6.3 (0.79)	12.4 (1.09)	4.5 (0.65)	6.8 (0.83)	7.1 (0.85)
T_2- Intercropping of moong bean *fb* pea *fb* moong bean	8.2 (0.91)	18.4 (1.26)	21.7 (1.33)	6.8 (0.83)	7.3 (0.86)	13.5 (1.13)	3.9 (0.59)	4.8 (0.68)	6.5 (0.81)
T_3- T_1 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	4.1 (0.61)	12.4 (1.09)	13.9 (1.14)	5.6 (0.74)	6.5 (0.81)	11.8 (1.07)	4.2 (0.62)	5.1 (0.76)	5.5 (0.74)
T_4- T_2 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0kg/ha in each season	5.3 (0.72)	11.7 (1.06)	14.1 (1.14)	6.2 (0.79)	7.4 (0.86)	13.1 (1.11)	3.7 (0.56)	4.1 (0.61)	4.9 (0.69)
T_5- Metribuzin 0.5 kg/ha in each season	3.4 (0.53)	6.1 (0.78)	8.3 (0.91)	3.4 (0.53)	5.9 (0.77)	10.5 (0.02)	3.3 (0.51)	3.8 (0.57)	4.5 (0.65)
T_6- Glyphosate 2.0 kg/ha once in a season	4.0 (0.60)	8.4 (0.92)	11.6 (1.06)	4.1 (0.61)	5.7 (0.75)	9.4 (0.97)	3.8 (0.57)	4.3 (0.63)	4.8 (0.68)
T_7- Two mechanical weeding per season	6.1 (0.78)	12.3 (1.08)	15.8 (1.19)	5.4 (0.73)	6.2 (0.79)	11.5 (1.06)	4.4 (0.64)	4.9 (0.69)	5.3 (0.72)
T_8- Weedy check	7.3 (0.86)	9.4 (0.97)	14.5 (1.16)	6.1 (0.78)	7.3 (0.86)	10.8 (1.03)	4.1 (0.61)	4.7 (0.67)	5.1 (0.70)
LSD (P=0.05)	0.07	0.10	0.10	0.07	0.08	0.09	0.06	0.07	0.07
Rabi									
T_1- Intercropping of cowpea *fb* pea *fb* cowpea	8.2 (0.91)	16.4 (1.21)	18.1 (1.25)	4.3 (0.63)	6.8 (0.83)	7.2 (0.85)	4.8 (0.68)	7.3 (0.86)	8.2 (0.91)
T_2- Intercropping of moong bean *fb* pea *fb* moong bean	9.2 (1.96)	16.8 (1.22)	17.5 (1.24)	6.2 (0.79)	7.3 (0.86)	8.4 (0.92)	3.9 (0.59)	6.2 (0.79)	7.3 (0.86)
T_3- T_1 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	6.3 (0.79)	12.3 (1.08)	15.8 (1.19)	5.5 (0.74)	9.3 (0.96)	10.3 (1.01)	4.2 (0.62)	5.3 (0.72)	6.9 (0.83)
T_4- T_2 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	7.5 (0.89)	14.8 (1.17)	16.9 (1.22)	5.9 (0.77)	12.8 (1.10)	14.4 (1.15)	4.3 (0.63)	6.1 (0.78)	7.2 (0.85)
T_5- Metribuzin 0.5 kg/ha in each season	5.2 (0.71)	6.8 (0.83)	9.2 (0.96)	6.0 (0.77)	7.4 (0.86)	9.2 (0.96)	3.2 (0.56)	5.3 (0.72)	5.4 (0.74)
T_6- Glyphosate 2.0 kg/ha once in a season	4.3 (0.63)	8.3 (0.91)	11.3 (1.03)	5.4 (0.73)	6.8 (0.83)	8.4 (0.92)	2.8 (0.44)	4.8 (0.68)	4.9 (0.69)
T_7- Two mechanical weeding per season	6.2 (0.79)	7.4 (0.86)	14.3 (1.15)	6.2 (0.790	8.4 (0.920	8.4 (0.92)	4.2 (0.62)	5.2 (0.71)	5.7 (0.75)
T_8- Weedy check	6.9 (0.83)	7.3 (0.86)	12.6 (1.10)	5.7 (0.75)	8.8 (0.94)	9.2 (0.96)	6.2 (0.79)	6.4 (0.80)	6.9 (0.83)
LSD (P=0.05)	0.08	0.10	0.11	0.07	0.09	0.09	0.06	0.07	0.08

Data in parentheses indicate the log_{10} transformed values, cfu-colony forming units, DAS-Days after spraying

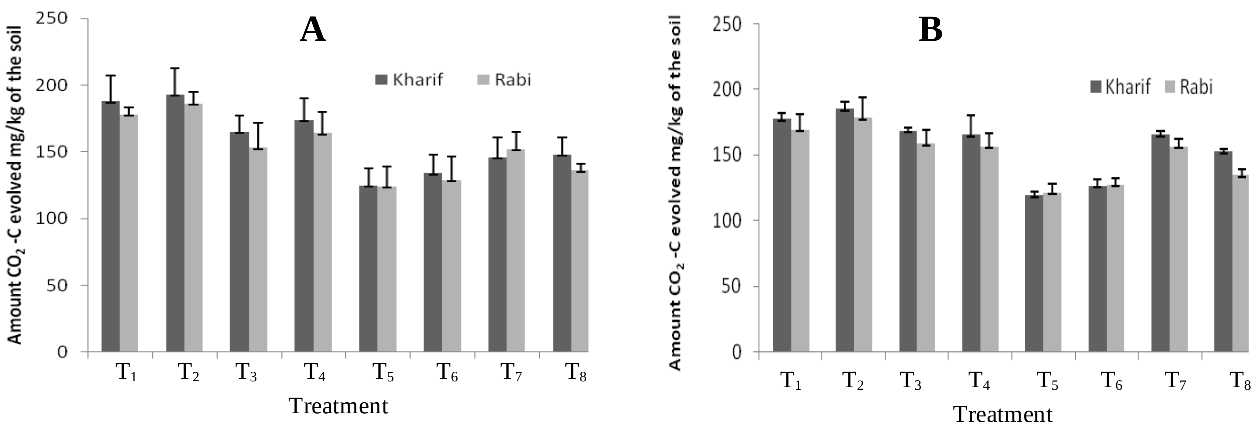
may lead to significant changes in the populations of microorganisms and their activities thereby influencing the microbial ecological balance in the soil (Saeki and Toyota 2004) and affecting the productivity of soils. The behaviour of herbicides in the soil has been studied now for several decades. When herbicides are applied to soil, they may exert certain side effects on non-target organisms.

Basal respiration rate was significantly more in cowpea-pea-cowpea and greengram-pea-greengram as intercropping system combined with and without herbicide treatment. Similar effect was observed by Tu (1991). Among the treatments, intercropping of greengram-pea-greengram showed more respiration rate (193mg/kg of CO_2-C) followed by intercropping of cowpea-pea-cowpea (188 mg/kg of CO_2 -C) during *Kharif* season in mango orchard. Similar trend was observed in citrus orchard (Fig. 1). LiJun *et al.* (2005) reported slight reduction in respiration rate during initial application of herbicides in soil. The potential indirect effect in the rhizospheres of intercropped species is enhanced nutrient mineralization due to change in soil organic matter decomposition rates, resulting from the addition of fresh organic matter. Thus, it can occur in the rhizosphere via root turnover and rhizodeposition. Fontaine *et al.* (2011) suggested that microorganisms use the energy from this fresh material to decompose soil organic matter in order to release organic N when inorganic N is limiting. P limitation has never been proven to provoke a priming effect, but it may be likely in ecosystems that are primarily P limited, such

Table 2. Effect of intercropping and herbicidal treatments on soil microbes in citrus orchard

Treatment	Soil microbial population (cfu/g soil)								
	Bacteria x10^6			Fungi x10^3			Actinomycetes x10^3		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Kharif									
T_1- Intercropping of cowpea *fb* pea *fb* cowpea	8.4 (0.92)	14.6 (1.16)	18.4 (1.26)	4.1 (0.61)	5.8 (0.76)	11.9 (1.07)	4.2 (0.62)	6.9 (0.83)	7.3 (0.86)
T_2- Intercropping of moong bean *fb* pea *fb* moong bean	8.2 (0.91)	13.5 (1.13)	21.3 (1.32)	5.7 (0.75)	7.3 (0.86)	12.3 (1.08)	3.7 (0.56)	4.5 (0.65)	6.9 (0.83)
T_3- T_1 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	4.3 (0.63)	8.1 (0.90)	12.4 (1.09)	5.1 (0.70)	6.5 (0.81)	10.8 (1.03)	4.1 (0.61)	4.5 (0.65)	6.1 (0.78)
T4- T_2 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	4.5 (0.65)	8.9 (0.94)	13.4 (1.12)	5.2 (0.71)	7.1 (0.85)	12.9 (1.11)	3.5 (0.54)	5.1 (0.70)	5.9 (0.77)
T_5- Metribuzin 0.5 kg/ha in each season	3.2 (0.50)	6.8 (0.83)	11.1 (1.04)	3.1 (0.49)	6.2 (0.79)	11.3 (1.05)	3.2 (0.50)	3.9 (0.59)	4.7 (0.67)
T_6- Glyphosate 2.0 kg/ha once in a season	3.8 (0.57)	7.4 (0.86)	10.9 (1.03)	4.3 (0.63)	8.7 (0.93)	9.8 (0.99)	3.7 (0.56)	4.2 (0.62)	4.9 (0.69)
T_7- Two mechanical weeding per season	6.3 (0.79)	11.2 (1.04)	13.8 (1.13)	5.1 (0.70)	6.2 (0.79)	8.9 (0.94)	4.3 (0.63)	4.9 (0.69)	5.6 (0.74)
T_8- Weedy check	7.3 (0.86)	9.4 (0.97)	14.6 (1.16)	5.4 (0.73)	6.9 (0.83)	10.5 (1.02)	4.2 (0.62)	4.5 (0.65)	5.3 (0.72)
LSD (P=0.05)	0.07	0.10	0.11	0.06	0.08	0.10	0.06	0.06	0.07
Rabi									
T_1- Intercropping of cowpea *fb* pea *fb* cowpea	8.2 (0.91)	16.4 (1.21)	17.3 (1.23)	4.4 (0.64)	6.8 (0.83)	14.8 (1.17)	4.8 (0.68)	6.8 (0.83)	7.5 (0.87)
T_2- Intercropping of moong bean *fb* pea *fb* moong bean	9.4 (0.97)	17.3 (1.23)	18.2 (1.26)	6.2 (0.79)	7.6 (0.88)	12.3 (1.08)	4.5 (0.65)	7.2 (0.83)	8.4 (0.92)
T_3- T_1 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	5.2 (0.71)	8.6 (0.94)	9.7 (0.98)	4.0 (0.60)	6.4 (0.80)	8.4 (0.92)	6.2 (0.79)	7.3 (0.86)	8.2 (0.91)
T4- T_2 + pendimethalin 1.0 kg/ha/ fluchloralin 1.0 kg/ha in each season	6.4 (0.80)	7.4 (0.86)	10.1 (1.00)	4.1 (0.61)	6.3 (0.79)	9.2 (0.96)	6.4 (0.80)	7.5 (0.87)	8.3 (0.91)
T_5- Metribuzin 0.5 kg/ha in each season	3.2 (0.50)	4.8 (0.68)	6.4 (0.80)	5.2 (0.72)	5.6 (0.74)	6.2 (0.79)	3.8 (0.57)	4.8 (0.68)	6.2 (0.79)
T_6- Glyphosate 2.0 kg/ha once in a season	2.8 (0.40)	3.4 (0.53)	4.8 (0.68)	5.3 (0.72)	5.7 (0.75)	6.8 (0.83)	3.2 (0.50)	4.9 (0.69)	6.3 (0.79)
T_7- Two mechanical weeding per season	6.8 (0.83)	7.4 (0.86)	8.4 (0.92)	4.8 (0.68)	6.8 (0.83)	7.8 (0.89)	4.6 (0.69)	5.3 90.72)	5.9 (0.77)
T_8- Weedy check	7.2 (0.85)	8.3 (0.91)	9.1 (0.95)	4.9 (0.69)	6.4 (0.80)	11.4 (1.05)	4.4 (0.64)	5.1 (0.70)	5.7 (0.75)
LSD (P=0.05)	0.08	0.08	0.09	0.07	0.08	0.09	0.06	0.07	0.08

Data in parentheses indicate the $\log_{10}$ transformed values, cfu-colony forming units, DAS-Days after spraying



(T_1-T_8: Treatments enforced as detailed in table 1)

Fig. 1. Intercropping and herbicidal treatments on soil respiration in A) mango and B) citrus orchard

as in the tropics. A positive priming effect (stimulation of SOM mineralization) should lead to the recycling of organic N and P and may ultimately enhance plant growth.

Based on our results, it is apparent that legume intercropping in mango and citrus orchard supported high microbial activity and further accelerated by organic matter incorporation. Results also indicated that the herbicidal treatments at the level tested were not drastic enough to be considered deleterious to soil microbial and soil respiration which are important to soil fertility. In addition to the more direct short-term supply of nutrients from decomposing leaf litter, nutrients can also be supplied indirectly from the mineralization of soil organic matter formed from the cumulative input of organic residues. Microbes play a key role in the process of organic matter decomposition and release of nutrients. Improvement in soil organic matter and microbial activity due to the addition of organic residues in tree-crop combination can lead to long-term sustainability of the tree-crop agroecosystem.

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