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EFFECT OF PEPPER-GARLIC INTERCROPPING SYSTEM ON SOIL MICROBIAL AND BIO-CHEMICAL PROPERTIES

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Abstract

The effects of pepper-garlic intercropping system on soil microorganisms i.e. bacteria, fungi, and actinomycetes; soil enzymes such as invertase, alkaline phosphatase, urease and catalase; soil chemical properties such as pH and electrical conductivity were investigated under plastic tunnel cultivation. Two intercropping models in which pepper were intercropped in standing normal garlic (sowing clove of cv. G026 for harvesting of scape and bulb) and green garlic (sowing bulb of cv. G064 for harvesting of green garlic). Intercropping, in which pepper was planted on each side of garlic row plantation were compared to mono-culture cultivation of pepper. Results showed that bacteria population significantly increased in the pepper plot intercropped with normal garlic, while actinomycetes were significantly enhanced in pepper plot intercropped with green garlic. Populations of fungi were significantly inhibited in pepper-green garlic intercropped plot. Intercropping of green garlic significantly increased the activities of invertase, alkaline phosphatase and catalase, while urease was promisingly higher in pepper plot intercropped with normal garlic. Soil pH was affected by intercropping and low level was measured in plot intercropped with normal garlic. Furthermore, soil EC was significantly higher in pepper plot intercropped with normal garlic. It is concluded that intercropping pepper with green garlic improved soil microbial and bio-chemical properties as compared to monocropping.

Introduction

Pepper (*Capsicum annum* L.) belongs to genus *capsicum* and family solanaceae is an important vegetable crop of China. It is believed to be originated in America and is now widely cultivated in most tropical and subtropical areas of the world (Tindall, 1992). Presently, China is the largest producer of pepper in the world (Anon., 2007; Diane, 2011). Pepper is considered to be an excellent source of compounds that stimulate immune system, prevent cardiovascular and cancer diseases, and also helps in delaying the aging process (Chuah *et al*., 2008; Podsedek, 2007). It is mainly used for adding flavour and imparting the pungency to cooked vegetables and meals. Pepper is mostly grown on a mono-cropping pattern under specialized protective structures in China, which carries many problems with it, such as deterioration of soil physicochemical properties and accumulation of toxic compounds. So mono-cropping system has negative impacts on the soil physical properties and structure, thus intercropping system is the better option to address these problems. Intercropping is the practice of growing two or more crops in close proximity in the same growing season. Intercropping is now becoming more important to improve soil quality and increase crop productivity (Li *et al.,* 1999). This cropping system is particularly significant in developing countries; where arable land is suppose to be limited. Various crops including fruits, vegetables, forages, and other field crops have been reported to be intercroped with peppers (Brian, 2010).

Garlic is known for its antimicrobial components mainly allicin. The exudates secreted by the rooting system of garlic can cause pronounce effects on soil structure and ecology. Furthermore, it resulted in some significant impact on the growth patterns, fruit yield and quality of intercropped plant. Hence intercropping of garlic with pepper can be productive in overcoming problems created during continuous cropping system. Studies showed that soil microbial diversity, soil enzyme activities and crop yield could be affected by land management practices (Acosta-Martinez *et al.*, 2010; Carney *et al.,* 2004). Soil enzyme is one of the major soil components present in a very nominal quantity in soil, but its role in improving soil quality can never be ignored (Huang, 1981; Li, 1981). The soil enzyme activities are related to positive trend of biochemical process, which can directly affect the soil productivity, economic ability and ecosystem performance (Dai & Bai, 1995; Shun & Tong, 2001). Soil enzyme activities, microbial population and soil nutrient contents were investigated under intercropping systems as compared to monoculture (Xie *et al.,* 2007; Willey, 1990). It is believed that enhanced nutritional uptake is not the only reason for yield increase under intercropping system, but many other unknown causes also affect it (Dessougi *et al.*, 2003). However, limited information is available regarding impact of garlic intercropping on soil microbial communities, enzyme activities, soil pH and electrical conductivity. Soil has a complex and unique environment, in which the biological activity is mostly controlled by microorganisms. Soil microorganisms play a critical role in nitrogen, sulphur and phosphorus cycles as well as ecosystem functioning by changing soil structure formation, organic matter decomposition, nitrogen fixation and toxin removal (Acosta-Martinez *et al.,* 2010; Karlen *et al.,* 1997; Gomes *et al.,* 2003). Research showed that the extent of soil microbial diversity is important for maintaining good quality of agricultural soil (Acosta-Martinez *et al.,* 2010; Garbeva *et al.,* 2004; Janvier *et al.*, 2007). On the other hand, microbial diversity of soil is affected by land management practices (Acosta-Martinez *et al.*, 2010;

Garbeva *et al*., 2004; Wieland *et al*., 2001). Composition of microbial communities in soil can be affected either directly by changing physiology of host plant or indirectly by altering patterns of root exudation (Marschner *et al.,* 2001; Van der Heijden, 1998). Soil enzymes derived primarily from soil microbial populations, plant root system and organic wastes indicate the potential to support biochemical processes involving decomposition of organic residues and nutrient cycling in soil (Lalande *et al.,* 2000; Casucci *et al.*, 2003). Studies demonstrated that soil enzyme activities could be used as a suitable indicator of soil quality (Gianfreda *et al.,* 2005; Acosta-Martinez *et al*., 2007). The objective of this research is to study the effect of garlic intercropping with pepper on dynamic changes in soil microbial population, enzyme activities, soil pH and EC as compared with mono-cropping.

Materials and Methods

Material and experimental design

Experimental site: The research was conducted in a plastic tunnel at Horticultural Experimental Station (N 34° 16', W 108° 4'), College of Horticulture, Northwest A & F University, Yangling, Shaanxi province, China. The physical and chemical properties of soil were analyzed as: electrical conductivity (409 µS cm^{-1}), pH (1:1 water) (7.46), organic matter (13.73 g kg^{-1}), total nitrogen (N) (1.73 g kg^{-1}), total phosphorus (P) (1.13 g kg^{-1}), total potassium (K) (14.35 g kg^{-1}), available N (91.7 mg kg^{-1}), available P (137.25 mg kg^{-1}) and available K (425.22 mg kg^{-1}).

Experimental details: Two garlic cultivars namely ‘Caijiapo Red Skin’ (G026) and ‘Gailiang’ (G064) were grown in the field during 2010 and these two cultivars were selected on the basis of their performance during autumn 2009 to spring 2010 in a previous study. Two intercropping models in which pepper were intercropped with standing normal garlic (sowing clove of cv. G026 for harvesting scape and bulb) and standing green garlic (sowing bulb of cv. G064 for harvesting of green garlic). During March 2011, pepper seedlings were intercropped with garlic plants using RCBD factorial design with three replications and three treatments. Each plot having a size of 3.5 × 1.2 m was set up for each treatment. In each plot pepper plants were planted at 30 cm P × P and 60 cm R × R distance. In intercropping pepper with normal garlic plots, three rows of garlic cloves were maintained in centre of the bed with each row contained 54 cloves. The garlic population was sandwiched by two parallel rows of pepper with each row comprised 11 plants. Similarly intercropping green garlic with pepper, each bed contained four rows of garlic bulbs with 67 garlic bulbs in each row. Again the garlic was sandwiched by 2 rows of pepper plants and each row having 11 plants. Mono-cropped pepper plants were grown representing control treatment. Before the transplantation of pepper plants, N and P fertilizers were applied at rates of 595 kg N and 595 kg P_2O_5 ha^{-1}. N and P were provided in form of ammonium hydrogen carbonate and calcium superphosphate, respectively. At fruiting stage, K fertilizer was applied as KNO_3 using fertigation method. Other standard agronomic practices i.e. irrigation, hoeing and weeding were equally performed in all the treatments.

Analysis

Soil sampling: Soil samples collected at a depth range of 0-15cm were analyzed after every 20 days after transplanting of pepper and every 30 days after uprooting of garlic. Soil samples were randomly taken at a distance of 5 cm from pepper plants and were then mixed treatment wise for each replication in order to make a composite sample. Soil samples were immediately transferred to the laboratory, where part of these samples were stored at 4^{o}C for soil microbial analysis and remaining samples were dried at room temperature 25-30^{o}C for soil enzyme analysis.

Soil microorganism: Population of soil microorganisms including bacteria, fungi and actinomycetes was determined by using standard dilution plate method according to colony forming units (cfu) as described by Fan & Li (1982). The bacteria, fungi and actinomycetes were incubated with beef broth peptone substrate, Gause No.1 substrate and potato dextrose agar (PDA), respectively. Three plates were measured for each parameter of soil sample.

Soil enzymes: Soil urease activity was determined by incubating 5 g soil sample with 10 mL of 10% urea solution for 24 h at 37°C. The formation of ammonium was determined by spectrophotometer at 578 nm and the activity was expressed as NH_4-N mg g^{-1} soil 24 per h (Hoffmann & Teicher, 1961). Soil invertase activity was determined by incubating 5g soil sample with 15 mL of 8% sucrose solution for 24 h at 37^{o}C. The suspension reacted with 3, 5-dinitrosalicylic acid and the absorbance was detected at 508 nm wavelength. Activity was expressed as glucose mg g^{-1} soil 24 per h (Frankenberger & Johanson, 1983). Similarly, 2g soil sample was incubated with 2mL of 0.3% H_2O_2 for 30 min at 30^{o}C for determining soil catalase activity. The suspension was titrated with 0.1 M L^{-1} $KMnO_4$ solution. Activity was expressed as 0.1 M L^{-1} $KMnO_4$ ml g^{-1} soil 30 per min (Johnson &Temple, 1964). Alkaline phosphatase activity was measured by incubating 1g soil with 4ml of 5% Na2RPO4 (R indicates benzene material) solution for 24h at 37°C and the formation of phenol was determined by spectrophotometer at 660nm and the activity was expressed as mg phenol·g^{-1} 24 per hour (Wan & Ping, 2004).

Measurement of soil pH and EC: Soil pH was measured by using a pH meter (PHS-3C, Shanghai Lida instrument factory, Shanghai, China) in a ratio of 10:10 (W/V) soil and water extract, after shaking for 30 min. Similarly, electrical conductivity (EC) measured in a 10:50 soil and water extract (w/v) by using an EC meter (LF 330, Wissenschaftlich-Technische, Weilheim, Germany) after waiting for 30 min. (Abouziena *et al.,* 2010).

Statistical analyses: Data were statistically analyzed by using SAS (Anon., 2001). The appropriate standard of deviation means were calculated .The means were compared using Least Significant Difference test (LSD) at p<0.05.

Results

Bacteria: A significant effect of intercropping was observed on population of soil bacteria (Fig. 1). A promising increasing impact on total number of soil bacteria was found after intercropping of pepper plants in standing garlic. All the garlic intercropped treatments showed gradually upward trend in number of soil bacteria after pepper plants were intercropped on 18th March, 2011; while it depicted a downward trend after uprooting of garlic on 28th April, 2011. Garlic bulb (green garlic intercropping) and garlic clove (normal garlic intercropping) were sown in standing pepper on 2nd August and 15th September, 2011 respectively and it attained maximum levels during October 2011, as compared to control treatment. The higher concentration of bacteria (21.998.4 x 10^{6}cfu g^{-1}) was measured on 10th October, 2011, while lower concentration (1.51x10^6 cfu g^{-1}) was recorded on 11th April, 2011.

Fungi: Population of soil fungi was significantly influenced by the intercropping (Fig. 2). A considerable decrease in soil fungi concentration was observed after intercropping of pepper on 18th March, 2011. The highest fungi concentration (8.237 x 10^{3}cfu g^{-1}) was found on 17th January, 2011 in control treatment, while lowest (0.22x10^{3}cfu g^{-1}) was recorded in treatments intercropped with green garlic on 22ndJune, 2011. Garlic bulb (green garlic intercropping) and garlic clove (normal garlic intercropping) were sown in standing pepper on 2nd August, 15thSeptember, 2011 respectively. After sowing of garlic it showed downward trend of fungi concentration during one year in plots intercropped with green garlic (G064), followed by normal garlic (G026) as compared to control.

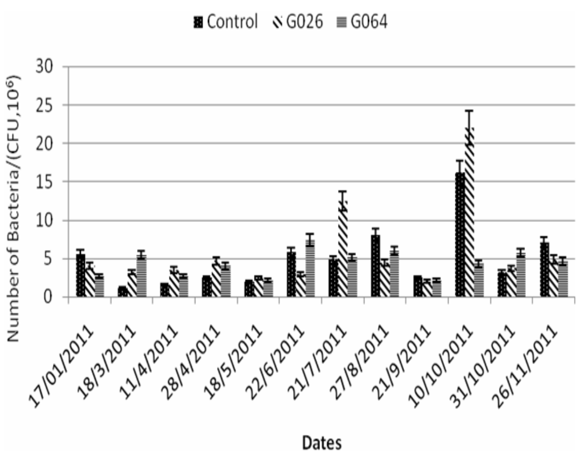

Fig.1. Effect of pepper-garlic intercropping on number of bacteria. Error bars present represent ± mean SD. $LSD_{(0.05)}$ =0.5757.

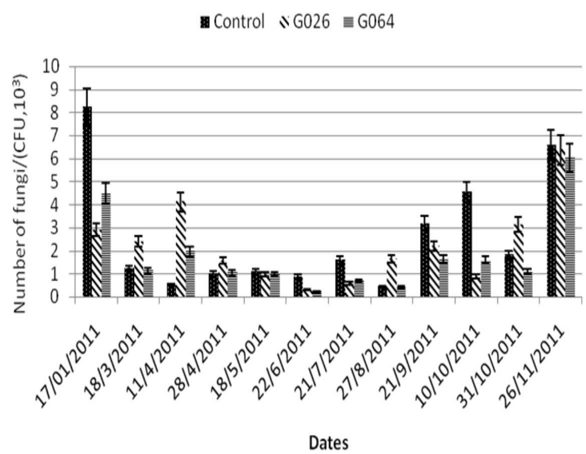

Fig. 2. Effect of pepper garlic intercropping on number of fungi. Error bars present represent ± mean SD. $LSD_{(0.05)}$ =0.9481.

Actinomycete: The dynamic changes in actinomycetes content depicted a positive effect of garlic intercropping on the soil actinomycetes population (Fig. 3). It was noted that highest concentration of actinomycetes (18.63 x 10^5 cfu g^{-1}) was found on 10th October, 2011 in plots intercropped with green garlic and after the intercropping of pepper in standing garlic on 18th March, 2011 the lowest concentration of actinomycetes (1.57x 10^5 cfu g^{-1}) was recorded on18th March, 2011 in control treatment. The graph revealed that after sowing of garlic bulb (green garlic intercropping) and garlic clove (normal garlic intercropping) on 2nd August and 15th September, 2011 respectively, a higher trend of actinomycetes concentration during one year were observed in green garlic intercropped plots.

Invertase: The soil invertase activities were significantly affected by intercropping (Fig. 4). When pepper plants were transplanted in standing garlic crop on 18th March, 2011 the invertase enzyme activity considerably increased and treatment intercropped with green garlic showed a higher activity of invertase (95.53 mg g^{-1}) on 11th April, 2011. Furthermore, after uprooting of garlic on 28th April, 2011 lowest value (40.53 mg g^{-1}) was recorded in plots intercropped with normal garlic. The invertase enzyme activity increased after sowing of garlic bulb (green garlic intercropping) and garlic clove (normal garlic intercropping) on 2nd August and 15th September, 2011 respectively in comparison to control.

Alkaline phosphatase: The alkaline phosphatase activities were affected by the intercropping practice as shown in Fig. 5. It was observed that after the intercropping of pepper in standing garlic on18th March, 2011, the highest alkaline phosphate activity (2.343mg g^{-1}) in comparison to control was recorded on 4th April, 2011 in plants intercropped with normal garlic.After uprooting of garlic on 28th April, 2011 low alkaline phosphatase (1.38 mg g^{-1}) activity was noted on 21stJuly, 2011 in plots intercropped with normal garlic. The graph showed an upward trend in alkaline phosphate activity after sowing of garlic bulb (green garlic intercropping) on 2nd August, 2011 and garlic clove (normal garlic intercropping) on 15th September, 2011. The alkaline phosphate activity was observed higher during one year in the plot intercropped with green garlic followed by normal garlic as compared to the control.

Urease: The urease activity was significantly affected by intercropping as compared to control (Fig. 6). It was observed that after the intercropping of pepper in standing garlic on 18th March, 2011, the highest urease activity (4.733mg g^{-1}) was recorded on the 10th October, 2011 in plots intercropped with normal garlic, while lowest (1.557mg g^{-1}) on 4th April, 2011 in the control. The graph depicted a positive trend in urease activity after sowing of garlic bulb (green garlic intercropping) on 2nd August, 2011 and garlic clove (normal garlic intercropping) on 15th September, 2011.

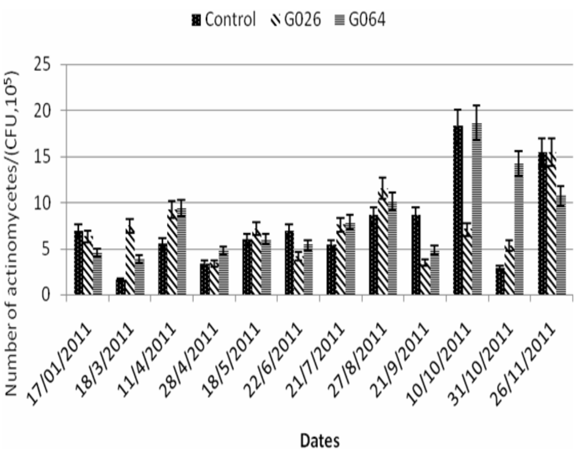

Fig. 3. Effect of pepper- garlic intercropping on number of actinomycetes.Error bars present represent ± mean SD.
$LSD_{(0.05)}$ =0.637.

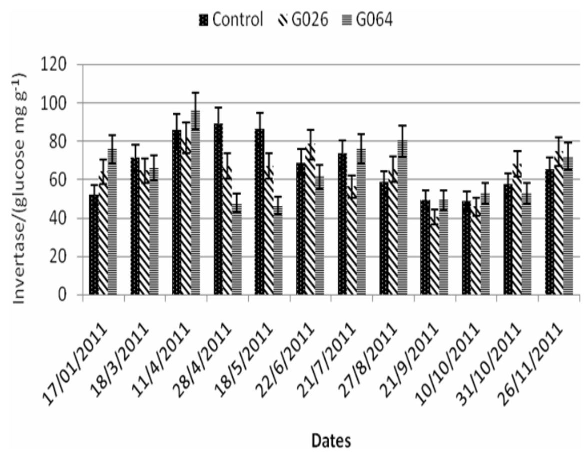

Fig. 4. Effect of pepper- garlic intercropping on invertase .Error bars present represent ± mean SD.
$LSD_{(0.05)}$ =10.91.

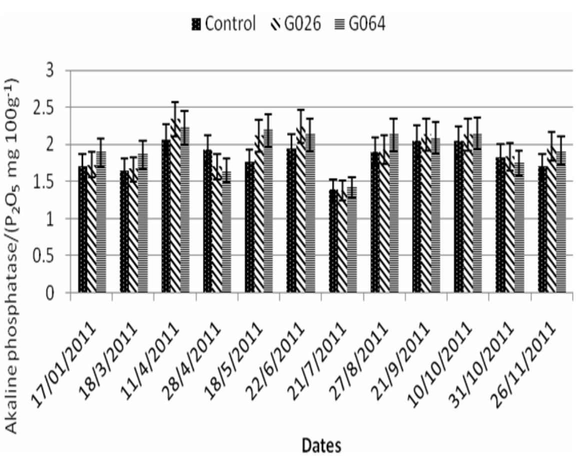

Fig. 5. Effect of pepper- garlic intercropping on alkaline phosphatase. Error bars present represent ± mean SD.
$LSD_{(0.05)}$ =0.4939.

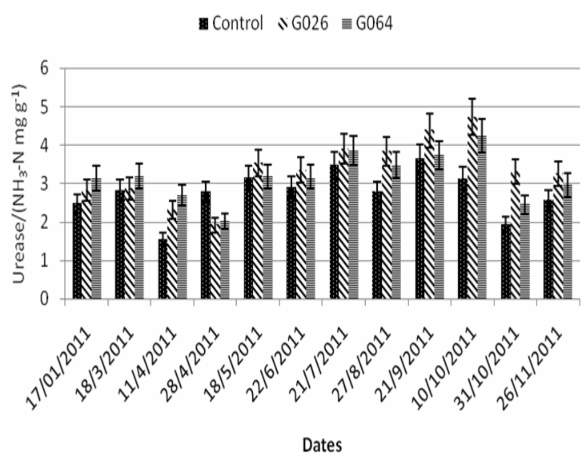

Fig. 6. Effect of pepper- garlic intercropping on urease. Error bars present represent ± mean SD.
$LSD_{(0.05)}$ =0.557.

Catalase: The catalase activity did not show significant difference among intercropped treatments (Fig. 7). It was noted that after the intercropping of pepper in standing garlic on 18th March, 2011 higher catalase activity (2.29ml g^{-1}) was recorded on 22nd June, 2011 in treatments intercropped with green garlic; while lowest catalase activity (1.553ml g^{-1}) was recorded on 17th January, 2011 in plots intercropped with green garlic *i.e.,* before intercropping of pepper. The graph showed a positive trend in catalase activity after sowing of garlic bulb (green garlic intercropping) and clove (normal garlic intercropping) on 2nd August and 15th September, 2011 respectively.

Soil pH: The soil pH significantly affected by intercropping (Fig. 8). Intercropped treatments were found with lower soil pH as compared to control. Before the intercropping of pepper in the standing garlic on 18th March, 2011 the highest soil pH (7.92) was recorded on 18th March, 2011 in plots intercropped with green garlic, whereas, lowest soil pH (7.53) was observed on 10th October, 2011 in treatments intercropped with normal garlic. The lower pH values were observed during one year in the plot intercropped with normal garlic as compared to the other treatments.

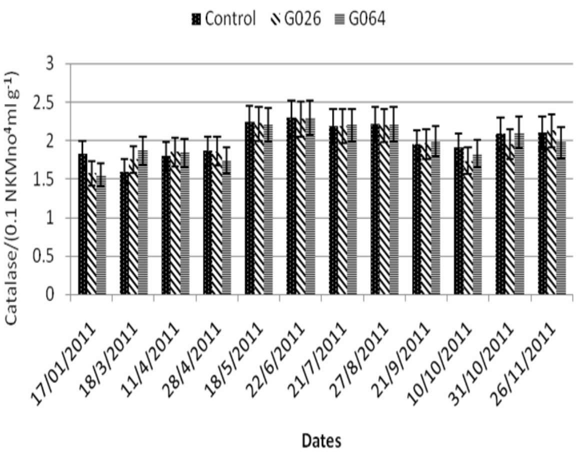

Fig. 7. Effect of pepper- garlic intercropping on catalase. Error bars present represent ± mean SD.
$LSD_{(0.05)}$ =0.1628.

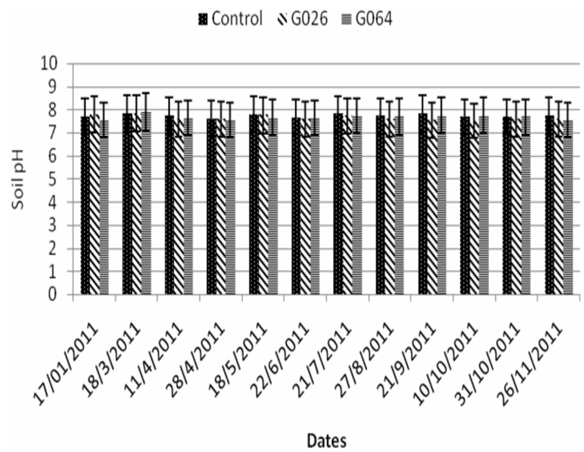

Fig. 8. Effect of pepper- garlic intercropping on soil pH. Error bars present represent ± mean SD.
$LSD_{(0.05)}$ = 0.0515.

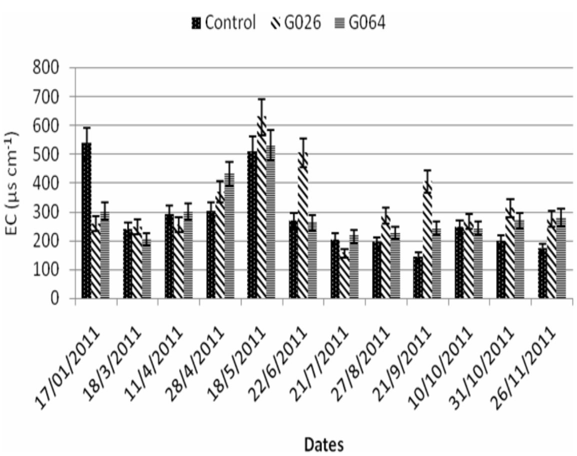

Fig. 9. Effect of pepper- garlic intercropping on soil EC Error bars present represent ± mean SD.
$LSD_{(0.05)}$ =32.24.

Electrical conductivity: Soil EC was significantly affected by intercropping (Fig. 9). An upward trend in EC was observed after the intercropping of pepper in standing normal garlic on18th March, 2011. The highest EC (629 µs cm^{-1}) in comparison to the control was recorded on 18th May, 2011 in treatments intercropped with normal garlic, while the lowest EC (145 µs cm^{-1}) was recorded on 21st September, 2011 in the control. The graph showed an upward trend in soil EC till 21st September, 2011 and then depicted a downward pattern during October and November. The higher EC was recorded during one year in the plot intercropped with normal garlic as compared to the other treatments.

Discussion

In present study, pepper–garlic intercropping markedly changed the population of soil microbial communities. Intercropping with garlic increased the concentration of bacteria and actinomycetes but decreased fungi in the rhizosphere as compared to mono-cropping. This may be due to the interaction of soil microorganisms and root exudates (garlic), which may affect soil microbial communities. Previous studies showed that the amount and kind of root exudates differ between plant species, and these differences can stimulate species-specific shifts in the soil microbial community (Uren, 2000). Root exudates contain root-specific metabolites that might have critical ecological impacts on soil macro and micro biota as well as on the whole plant. Through the exudation of a wide variety of compounds, roots affect the soil microbial community in their immediate vicinity, supporting beneficial symbioses, alter the chemical and physical properties of the soil, and inhibit the growth of competing plant species (Bertin *et al.*, 2003). Our results are also in agreement with Keswani *et al*., (2003), who stated that intercropping increased the bacterial rhizosphere soil ratio of maize and soybean, whereas fungi rhizosphere soil ratio showed a decrease in both cases when compared to sole crop condition. Functional diversity and metabolic activity of soil microbial community improved under intercropping. Intercropping could significantly increase the quantity of soil bacteria in both maize and peanut root areas (Zhang *et al.,* 2009; Lixuan *et al*., 2007). As demonstrated by Wu *et al*., (2010) that intercropping aromatic plants in sandy soil in pear orchard had good regulatory effects on the soil microbial quantity. In another study conducted by Chai *et al*., (2005) that intercropping significantly enhanced the number of actinomyctes and total microbial population in the rhizosphere as compared to monoculture cropping.

Soil enzyme activity is critically important for soil quality and can provide basic indications for changes in metabolic capacity and nutrient cycling due to management practices (Saha *et al*., 2008). Some previous studies showed that continuous monoculture cropping pattern was detrimental to soil enzyme activities and significantly decreased under this cropping system (Wu *et al.*, 2006). The present results are in agreement with Zhao *et al*., (2011), who explored that invertase, urease, catalase and alkaline phosphatase activities during the whole growth period of garlic- cotton and wheat-cotton intercropping systems were significantly higher as compared to cotton monoculture. Similar results were also reported by Lei *et al*., (2012), who observed higher invertase, urease, alkaline phosphatase and catalase activity in intercropped treatments. These findings are supported by Jiang *et al*., (2010) who found that intercropping system could improve the soil enzyme activity. Similar findings were also reported by (Xingang *et al*., 2011), who explored that intercropping cucumber with onion or garlic increases soil urease and catalase activities. Similar improvement in soil quality by the green manure legumes was reported by Shah *et al*., (2011).These findings are also in agreement with Cai *et al*., (2010) who reported that the activities of urease, phosphatase and catalase were all enhanced significantly after intercropping. These findings are also supported by Yang & Fz (2011) who reported that intercropping cucumber with Chinese onion cultivars significantly increased enzyme activities. These results are also supported by Han *et al.*, (2013) that decomposed garlic stalk had good impact on soil enzymes activities. Similarly, Wan & Ping (2004) found that the content of catalase , urease, invertase and alkaline phosphatase were more in intercropped soil. Similarly, Kuang *et al*., (2010) reported that intercropping had significant effect on catalase activity.

Plants produce and release secondary compounds and unique combinations of compounds through leaf leachates, leaf litter, leaf volatiles, and root exudates. The production and release of these compounds, however, can be affected by environmental conditions. Importantly, many of these compounds have the potential to change the chemical composition of the substrates in which plants grow. Some plants species e.g. *Vicia faba* when grown in phosphorus poor conditions, acidifies its rhizosphere with malate and citrate, substantially lowering the pH of growth media (Jeffrey & Callaway, 2010). In present study intercropping caused a positive effect on soil pH. The results are in line with Singh, (1995) who observed that pH values were recorded lower in *Prosopis juliflora* and *Leptochloa fusca* based intercropping system as compared to monocropping. These results are in agreement with Emmanuel *et al*., (2010) who reported that intercropping annual ryegrass with pinto beans decreased the soil pH. In another study soil pH was significantly reduced in cassava- soybean based intercropping system (Makinde *et al.*, 2006). These findings are also supported by Bughio *et al*., (2013) that soil pH decreased with increasing concentration of leaf litter aqueous extract of *Eucalyptus camaldulensis.* EC is an important soil quality indicator affected by crop sequence and tillage (Liebig *et al*., 2004). In present study, intercropping significantly increased soil EC and these results are supported by (Zhang *et al*., 2009) who studied that in maize/peanut intercropping electrical conductivity was increased as compared to mono-cropping. These finding are also supported by Hua *et al*., (2005) who reported that Chinese fir-crop combinations increased soil electrical conductivity as compared to monocropping.

Conclusion

Actinomycetes population was higher in treatment intercropped with green garlic (cv. G064), while bacteria was higher in treatment intercropped with normal garlic (cv.G026) as compared to the control. Population of soil fungi were found lower in pepper plot intercropped with green garlic. Soil enzymes i.e. sucrase, alkaline phosphatase and catalase enhanced in pepper plots intercropped with green garlic, while urease enzyme was higher in treatment intercropped with normal garlic. Soil pH was higher in control treatment as compared to intercropped treatments, while soil EC was recorded higher in plot intercropped with normal garlic. It is concluded that intercropping pepper with green garlic improved soil microbial and bio-chemical properties as compared to monocropping.

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