

EFFECT OF INTERCROPPED GARLIC (*ALLIUM SATIVUM*) ON CHLOROPHYLL CONTENTS, PHOTOSYNTHESIS AND ANTIOXIDANT ENZYMES IN PEPPER

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Abstract

The effects of intercropped garlic (*Allium sativum*) on chlorophyll a, chlorophyll b, total chlorophyll content, leaf photosynthetic rate and antioxidant enzymes were investigated in pepper during two years study from 2011 to 2012. Physico-chemical analysis of pepper intercropped with 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) were compared to mono-culture cultivation of pepper. Results showed that chlorophyll a, total chlorophyll content, net photosynthetic rate and catalase activity (CAT) were significantly enhanced at $p < 0.05$ in the pepper intercropped with normal garlic, while chlorophyll b and peroxidase (POD) were significantly enhanced at $p < 0.05$ in the pepper intercropped with green garlic. Polyphenol oxidase (PPO) was inhibited by intercropping and high level of (PPO) activity observed in control. It can be concluded from this study that intercropping pepper with normal garlic increased chlorophyll content, photosynthetic rate and antioxidant enzymes activity as compared to other treatments.

Introduction

Pepper (*Capsicum annuum* L.) belongs to solanaceae family and is a major and economically profitable vegetable for the growers in China. It was originated in America and is now widely cultivated in most tropical and subtropical areas of the world (Tindall, 1992). Out of 20 wild species, five are cultivated, with *Capsicum annuum* being the most widely grown. China is the largest producer of pepper in the world (Anon., 2007; Diane, H. 2011). Pepper contains such chemical compounds that can stimulate immune system, prevent cardiovascular and cancer diseases and delay aging process (Chuah *et al.*, 2008; Podsedek, 2007). It is rich source of vitamin A and C and is mainly used for adding flavor and imparting pungency to cooked vegetables and other dishes. The continuous mono-cropping pattern of pepper crop under protected structures in China has resulted in various problems i.e. deterioration of soil physicochemical properties and accumulation of toxic compounds. So intercropping is considered to be a better option for avoiding these deleterious effects of continuous cropping. It is a crop management system that involves co-culture of two or more economically beneficial species for at least a portion of their respective productive cycle and planted sufficiently close to each other so that inter specific competition occurs (Sulliva, 2003; Dugje, 2004). Intercropping pattern can also be beneficial in some other perspectives such as soil erosion control, reduced leaching of nutrients, soil fertility, reduced crop losses due to less proportion of weeds and pest or disease control, balanced distribution of labor and higher economic returns than sole cropping (Odo & Futuless, 2002; Alamu *et al.*, 2002; Blaser *et al.*, 2007; Khan *et al.*, 2012).

Garlic is known for its antimicrobial components mainly allicin. The exudates secreted by the rooting system of garlic have a pronounced effect on the soil structure and ecology (Xuemei *et al.*, 2012). It holds a significant impact on the plant growth patterns, fruit yield and quality of the other intercropped crop. Hence intercropping of garlic with

pepper will be advantageous in overcoming continuous cropping problems. Allelochemicals are released to the environment in an appreciable amount through root exudates, leaf leachates, roots and other degrading plant residues, which can affect and modify the growth and development of plants, including germination and early seedling growth (Rice, 1984). The quantity and quality of root exudates depends on plant species, cultivar, developmental stage and environmental factors (Uren, 2000). Allelochemicals are considered to alter a variety of physiological and biochemical processes, thus it's often difficult to draw a line between primary and secondary effects. There are increasing evidences that allelochemicals have significant effect on cell division, ion and water uptake, phytohormone metabolism, respiration, photosynthesis, enzyme function, as well as gene expression (Singh & Thapar 2003; Inderjit & Duke 2003; Belz & Hurle 2004). Recently, the role of the antioxidant system in the plants as a response to environmental stress has received wide attention. Although some studies showed that autotoxins or allelochemicals can increase ion leakage by altering membrane permeability (Yu and Matsui, 1997). Our understanding regarding the role of antioxidant enzymes in response to allelopathic agents is very limited and previously concerned studies have not yielded conclusive results (Jing *et al.*, 2003). The effects of root exudates on physiological variables have scarcely been examined (Yu & Matsui, 1997). The objective of the present study was to investigate the effect of garlic root exudates on enzyme antioxidant activity, photosynthesis and chlorophyll content of pepper in the pepper - garlic based intercropping system.

Materials and Methods

Plastic tunnel experiment: 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 of scape and bulb) and 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 (replication) 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 15 x 6 cm apart and each row contained 54 cloves. The garlic cloves were sandwiched by two parallel rows of pepper with each row comprise of 11 plants. On the other hand intercropping green garlic with pepper, each bed contained four rows of garlic bulbs with 67 garlic bulbs in each row. Again the garlic bulbs were sandwiched by two rows of pepper plants and each row having 11 plants. Sole pepper plants were grown representing control treatment.

$$Ca \text{ (mg/L)} = 12.7 \times D663 - 2.59 \times D645$$

$$Cb \text{ (mg/L)} = 22.9 \times D645 - 4.67 \times D663$$

$$CT \text{ (mg/L)} = Ca + Cb = 20.3 \times D645 + 8.03 \times D663$$

$$\text{Chlorophyll content (mg/g)} = \frac{C \text{ (mg/L)} \times \text{total content of extract solution (ml)} \times \text{dilution factor}}{\text{Fresh weight of leaf (g)}} \times 1000$$

Plant enzymes: Five pepper plants were randomly sampled from each treatment for measuring plant enzyme activities. The plant samples were rinsed with distilled water and dried with absorbent paper, cut into 0.5cm² pieces and frozen in liquid nitrogen for several minutes and finally stored at -80°C. Enzyme solutions were extracted according to the modified method of Guo *et al.*, (2004). The frozen pepper leaves from each treatment were homogenized using mortar and pestle with 0.05M sodium phosphate buffer (pH 7.8). The homogenate was then centrifuged at 10000r/min for 20min and the supernatant was used for analyzing peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO) activities. All the above steps were carried out at 4°C. The activities of protective enzyme were determined as mentioned by Gao (2000). Peroxidase (POD), catalase (CAT) and polyphenol oxidase (PPO) activities were expressed as absorbance changes at 470nm, 240nm and 410nm per minute per g FW, respectively.

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

Results

Effect of intercropped garlic on chlorophyll a of pepper: A significant positive effect of garlic intercropping was recorded on chlorophyll a content in pepper leaves (Fig. 1). After intercropping of pepper on

Photosynthesis measurement: Photosynthetic rate was measured selecting the second fully developed leaf from top of the pepper during 9:00-11:00 am, using LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). Five plants of uniform shape and size were selected from each treatment for measurement and it was repeated for all three replicates. The net photosynthetic rate ($\mu\text{mol m}^{-2}\text{s}^{-1}$) was measured at a leaf temperature range of 25.5±2°C, and relative humidity (RH) of 45% in the leaf chamber (Zhang *et al.*, 2011).

Chlorophyll content: The chlorophyll estimation was conducted following the method of Ranganna (1986). The fresh leaf sample (0.1g) was mixed with 10 ml of 80% (V/V) acetone and kept in dark at room temperature for 24 hours until the leaves turned completely white. Absorbance of the solution for chlorophyll a and b was read at 663nm and 645nm wavelengths, respectively using the spectrophotometer (UV-3802, UNIC, Shanghai, China). The chlorophyll a and b were calculated using following formula:

March 18, 2011, all the garlic treatments showed a gradual upward trend in chlorophyll a of pepper; whereas it showed downward trend when garlic was uprooted on 28th April, 2011. The garlic bulb of G064 (green garlic intercropping) was sown in standing pepper on 2nd August, 2011, while garlic cloves of G026 cultivar (normal garlic intercropping) on 15th September, 2011 and it showed upward trend in chlorophyll a of intercropped treatments during November, 2011. Higher chlorophyll a content (1.20 mg·g⁻¹) was observed on 22nd June, 2011 in plants intercropped with garlic clove (normal garlic intercropping), while low chlorophyll a content (0.29mg·g⁻¹) was observed on 20th July, 2011 in plants grown with garlic bulb (green garlic intercropping).

Effect of intercropped garlic on chlorophyll b of pepper: A significance effect of garlic intercropping on chlorophyll b content of pepper was observed as depicted in Fig. 2. An increasing pattern of chlorophyll b content was recorded after intercropping of pepper on 18th March, 2011; whereas, a downward trend in all treatments were observed after uprooting of garlic on 28th April, 2011. Higher chlorophyll b content (0.86 mg·g⁻¹) was observed on 22nd June 2011 in the field intercropped with garlic bulb G064 (green garlic intercropping), while low chlorophyll b content (0.12mg·g⁻¹) was recorded in the plot intercropped with garlic clove G026 (normal garlic intercropping) on 20th July, 2011. The garlic bulb (green garlic intercropping) was sown in standing pepper on 2nd August 2011, while garlic clove (normal garlic intercropping) on 15th September, 2011 and it showed upward trend of chlorophyll b in pepper with green garlic followed by normal garlic as compared to control.

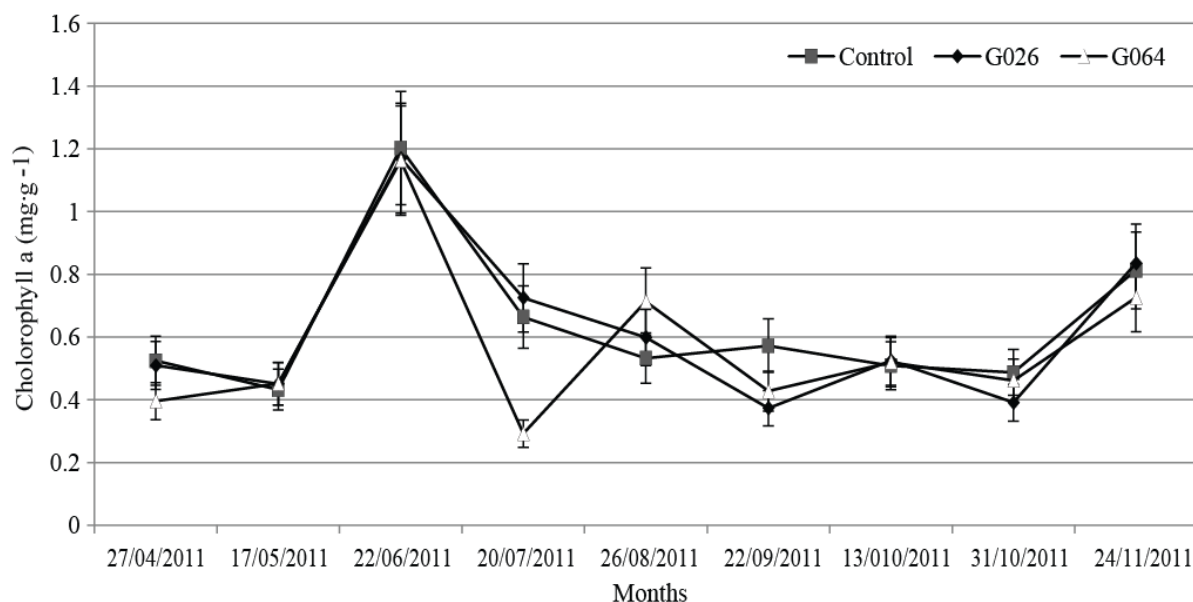


Fig. 1. Effect of intercropped garlic on chlorophyll a of pepper. Error bars present as the standard deviation of the mean.

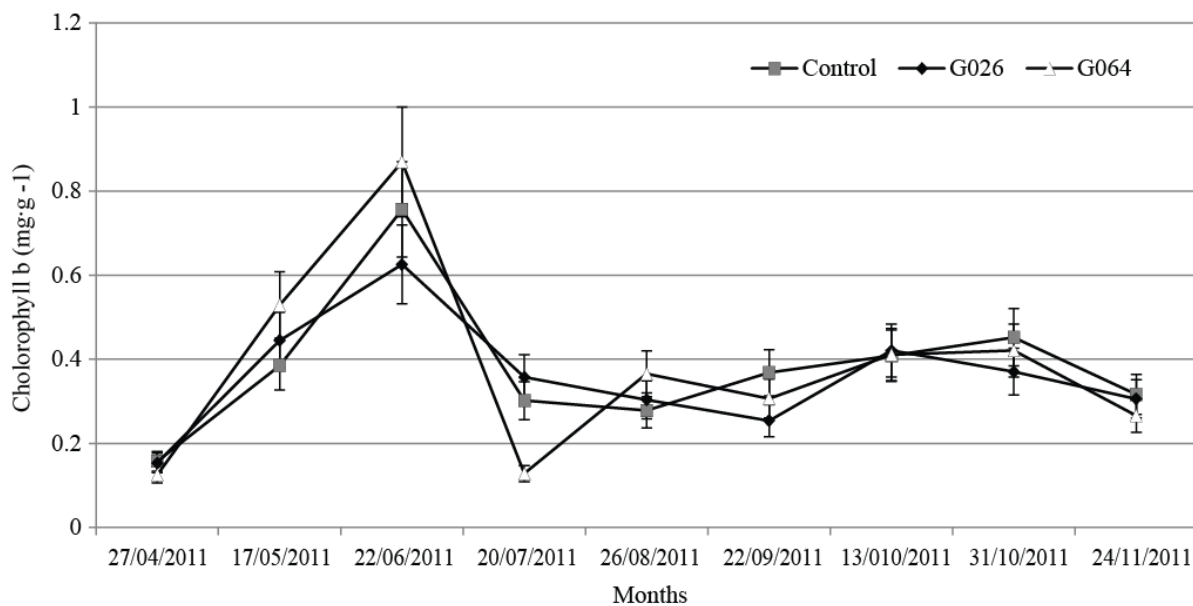


Fig. 2. Effect of intercropped garlic on chlorophyll b of pepper. Error bars present as the standard deviation of the mean.

Effect of intercropped garlic on total chlorophyll content of pepper: The dynamic change in total chlorophyll content of pepper due to garlic intercropping presented in Fig. 3 and it showed that intercropping had a significant effect on total chlorophyll content. After uprooting of garlic on 28th April, 2011 the higher total chlorophyll content ($2.03\text{mg}\cdot\text{g}^{-1}$) and lower total chlorophyll content ($0.420\text{mg}\cdot\text{g}^{-1}$) were found on the 22nd June, 20th July, 2011 respectively in garlic bulb G064 (green garlic intercropping) intercropped treatments. The graph showed that after sowing of garlic bulb (green garlic intercropping) on 2nd August, 2011 and garlic clove (normal garlic intercropping) on 15th September 2011, a higher trend of total chlorophyll content in all treatments

during one year were observed in normal garlic as compared to other treatments.

Effect of intercropped garlic on net photosynthetic rates of pepper: The net photosynthetic rate of pepper plants were significantly affected by intercropping (Fig. 4). The photosynthetic rate increased after garlic bulb (green garlic intercropping) and garlic clove (normal garlic intercropping) were sown in standing pepper on 8th August, 2011 and 15th September 2011, respectively. The highest net photosynthetic rate ($38.55\mu\text{mol m}^{-2} \text{s}^{-1}$) was observed on 8th October, 2011 in the field intercropped with garlic clove G026 (normal garlic intercropping), while lowest rate ($19.80\mu\text{mol m}^{-2} \text{s}^{-1}$) was recorded in control on 4th April, 2011.

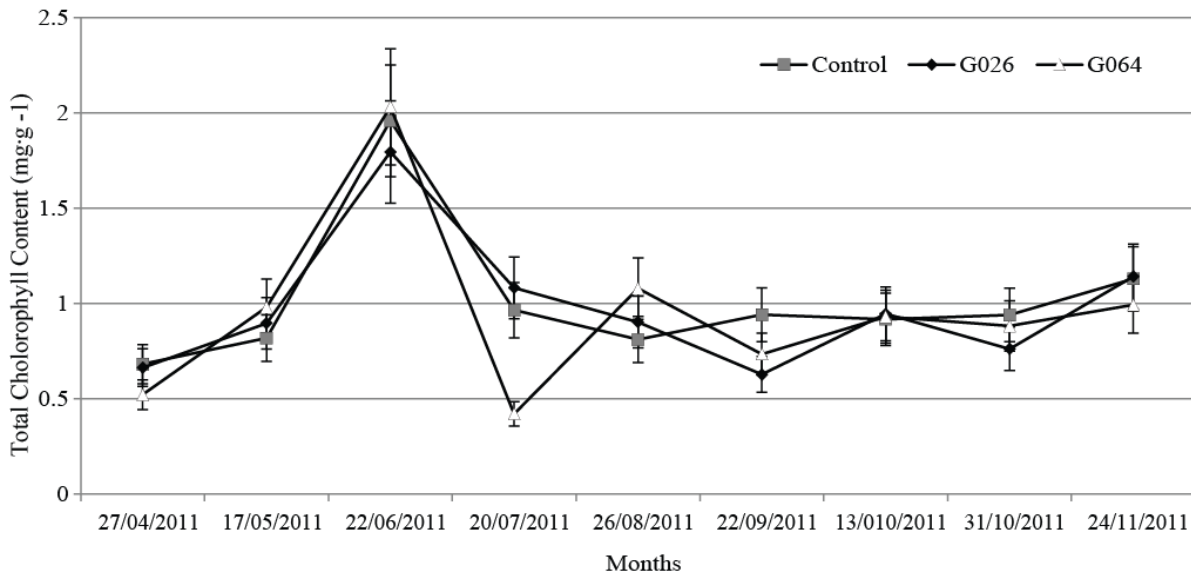


Fig. 3. Effect of intercropped garlic on total chlorophyll content of pepper. Error bars present as the standard deviation of the mean.

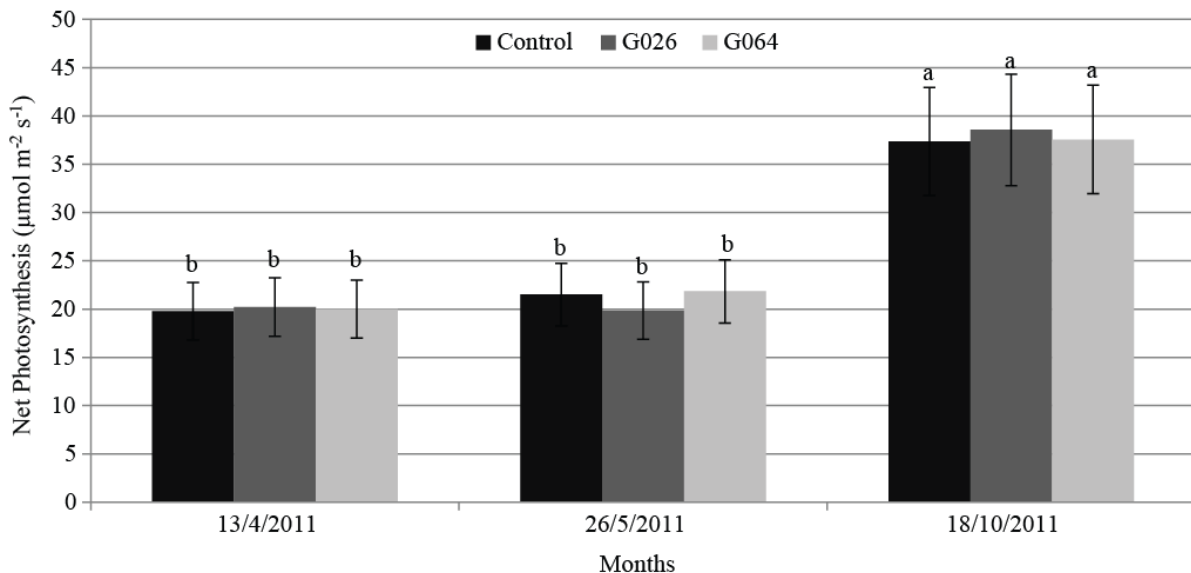


Fig. 4. Effect of intercropped garlic on net photosynthetic rate of pepper. Error bars present as the standard deviation of the mean. Different letters above the histograms indicate significant differences at 0.05 level (ANOVA and LSD test).

Effect of intercropped garlic on catalase enzyme activities ($\mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) of pepper: The intercropping had significantly affected the catalase enzyme activities of pepper leaves (Fig. 5). It was observed that after intercropping of pepper in standing garlic on 18th March, 2011, the highest CAT activity ($84.64 \mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) was recorded on the 22nd June, 2011 in plants intercropped with garlic clove G026 (normal garlic intercropping) as compared to control. Furthermore, after uprooting of garlic on 28th April, 2011, lowest CAT ($22.60 \mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) activity was noted on 20th July, 2011 in pepper plants intercropped with garlic bulb G064 (green garlic intercropping). The graph showed an increasing trend in CAT activity after sowing of garlic bulb (green garlic intercropping) on 2nd August, 2011 and garlic clove (normal garlic intercropping) on 15th September.

Effect of intercropped garlic on peroxidase (POD) enzyme activities ($\mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) of pepper: The POD activity of pepper was significantly improved by garlic intercropping as compared to control (Fig. 6). After the intercropping of pepper in standing garlic on 18th March, 2011 highest POD activity ($2288.71 \mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) was recorded on 27th April, 2011 in garlic bulb G064 (green garlic intercropping), whereas, lowest POD activity ($562.13 \mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) was noted on 22nd September, 2011 in pepper intercropped with garlic clove G026 (normal garlic intercropping). The graph depicted that after sowing of garlic bulb G064 (green garlic intercropping) on 2nd August, 2011 and garlic clove G026 (normal garlic intercropping) on 15th September, 2011, there was a downward trend in POD activity of older leaves.

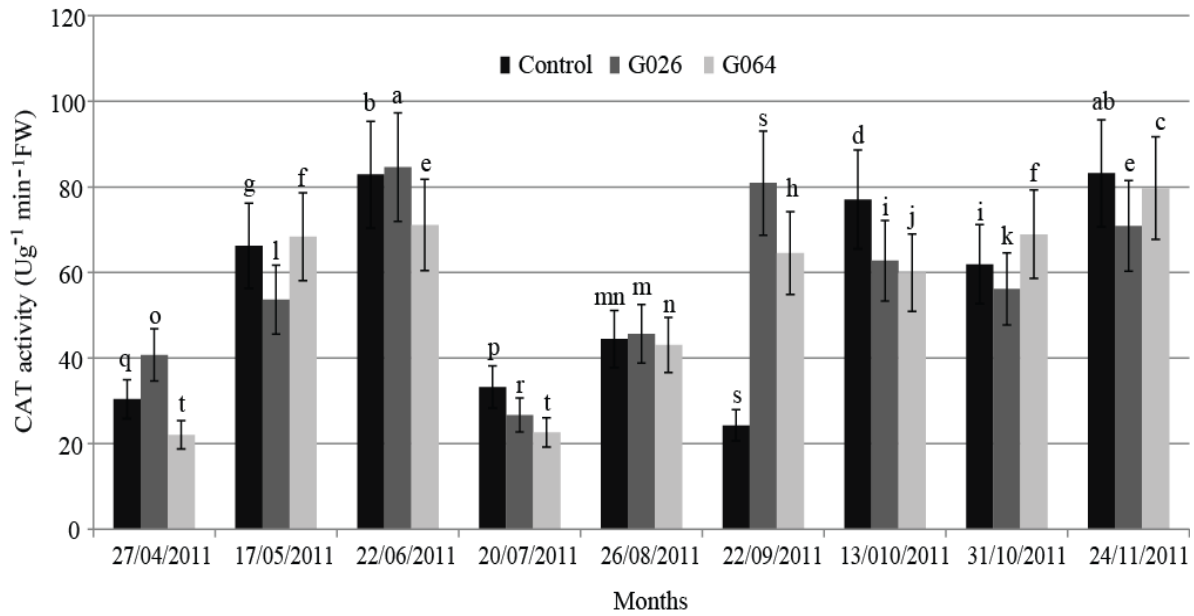


Fig. 5. Effect of intercropped garlic on catalase enzyme activities of pepper. Error bars present as the standard deviation of the mean. Different letters above the histograms indicate significant differences at 0.05level (ANOVA and LSD test).

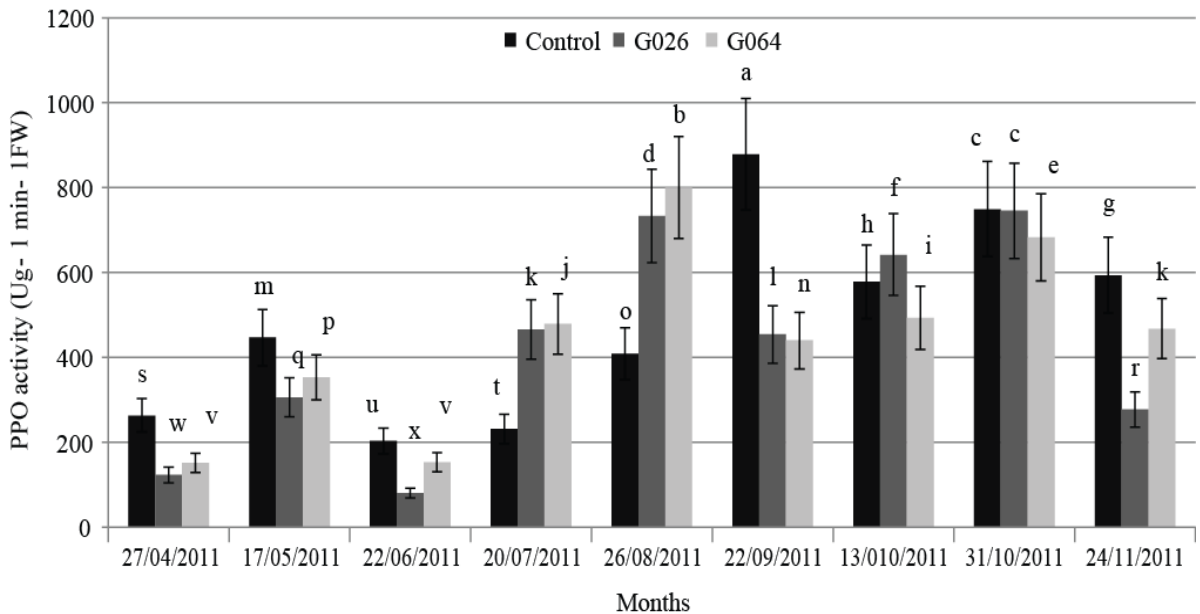


Fig. 6. Effect of intercropped garlic on peroxidase (POD) enzyme activities of pepper. Error bars present as the standard deviation of the mean. Different letters above the histograms indicate significant differences at 0.05level (ANOVA and LSD test).

Effect of intercropped garlic on polyphenol oxidase (PPO) enzyme activities ($\mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) of pepper:

PPO activity was significantly decreased in all the intercropped treatments as compared to the control (Fig. 7). It can be depicted that intercropping strongly inhibited PPO activity of the pepper leaves. The highest PPO activity ($878.22\mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) was recorded on the 22nd September, 2011 in control. After uprooting of garlic on 28th April, 2011 lowest PPO activity ($80\mu\text{g}^{-1} \text{min}^{-1}\text{FW}$) was noted on 20th July, 2011 in pepper intercropped with garlic clove G026 (normal garlic intercropping).

Discussion

Chlorophylls are the core component of pigment-protein complexes which play a major role in the photosynthesis. Any variation in Chlorophyll content is expected to bring change in photosynthesis rates. In our experiment it was found that chlorophyll content of pepper plants was significantly improved by garlic intercropping and it might be due to better availability of plant nutrients and higher light use efficiency in intercropped treatments. Previous studies reported that higher chlorophyll content (chlorophyll a + chlorophyll b)

was found in ginger (*Zingiber officinale*)/areca nut (*Areca catechu*) (Sankar & Swamy, 1988), and higher light use efficiency was measured in intercropped oats and vetch (*Vicia sativa*) than in monocropping (Ercoli *et al.*, 1997). These results are supported by Zhou *et al.*, (2007) that garlic root exudates significantly increased the chlorophyll content of the tested vegetables due to better absorption of nutrients in intercropped treatments. Bhatt *et al.*, (2008) reported that due to better growth and light interception the accumulation of chlorophyll a and b were higher in the leaves of crop species grown in intercropping system. These inferences are in line with the results of Massimo & Mucciarelli, (2003) that intercropped peppermint plants showed higher values of chlorophyll a and b as well as total chlorophyll contents. These results are in agreement with Otusanya *et al.*, (2008), who observed that chlorophyll b of *Capsicum annum* was significantly effected in the older plants, while chlorophyll b and total chlorophyll of *Lycopersicon esculentum* were also significantly enhanced. These findings are contrary to those reported by Bughio *et al.*,

(2013) that *Eucalyptus* leaf litter and leaf litter aqueous extract significantly reduced the chlorophyll content.

Photosynthesis is the basic physico-chemical process for plant growth, by which plants, algae and photosynthetic bacteria use light energy to drive the synthesis of organic compounds. Recent studies showed that allelochemicals also significantly influenced photosynthesis like other environmental factors. In this study net photosynthetic rate was significantly affected by intercropping and upward trend in photosynthetic rate might be due to increase in chlorophyll content of the intercropped treatments. These results are supported by Zhou *et al.*, (2007), who studied that garlic root exudates increased the chlorophyll content to enhance the absorption of light energy of tomato and hot pepper and which resulted in improved photosynthetic rate. Similar results were also reported by Olga *et al.*, (2007) that when tomato plants intercropped with marigold resulted in higher ($p \leq 0.05$) net photosynthetic rate and chlorophyll content as compared to control.

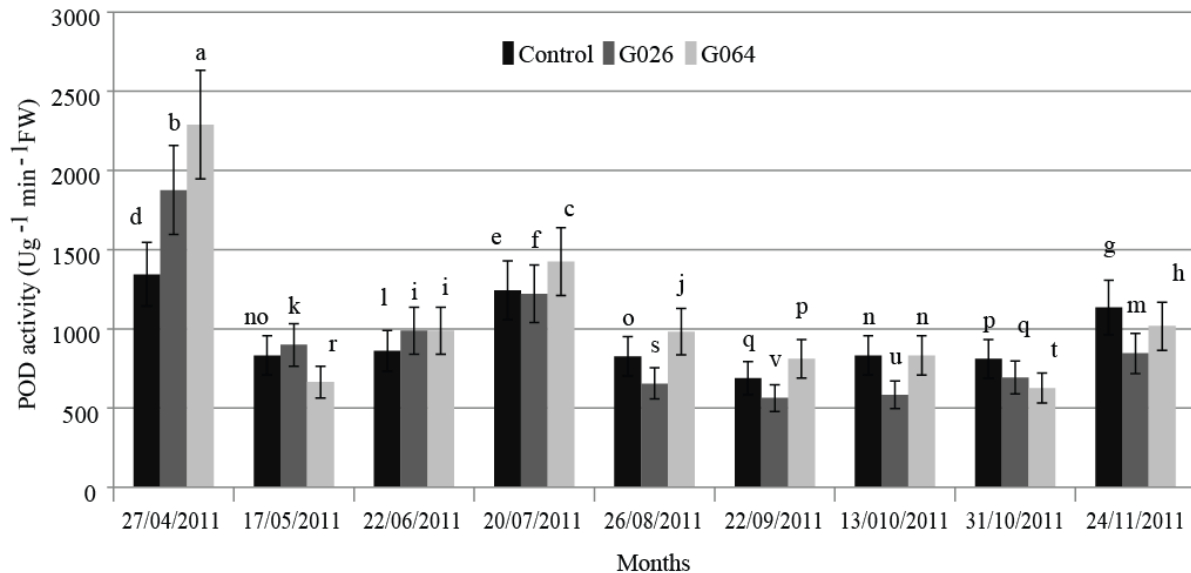


Fig. 7. Effect of intercropped garlic on polyphenol oxidase (PPO) enzyme activities of pepper. Error bars present as the standard deviation of the mean. Different letters above the histograms indicate significant differences at 0.05 level (ANOVA and LSD test).

Catalase is an enzyme of the anti-oxidant system and it prevents accumulation of H_2O_2 in the cells. It has been demonstrated that CAT activity can be induced in plant species (Junmin & Jin, 2010). In our study the (CAT) activity in pepper leaves was significantly improved by the garlic intercropping in pepper and it might be due to allelochemical which might have good impact on it. These results are supported by Li *et al.*, 2005 that the root, leaf and stem extracts of *Mikania micrantha* at both high and low concentrations significantly affected the CAT activity of the *Coix lacryma-jobi* seedlings, suggesting that the water extracts could generate oxidative stress and the cells presented a positive endogenous protective effect to cope with the increased concentration of H_2O_2 . Thus, the water extracts of *Mikania micrantha* might contain some allelochemicals

that can affect CAT activity. These results are complementary to the findings of Dong *et al.*, (2008) that concentration of 20 mg.ml^{-1} of garlic bulb aqueous extracts promoted POD and CAT activities, while higher concentrations inhibited POD and CAT activities. Similar results were also reported by Tong *et al.*, (2007) that garlic extracts at concentration of 0.02 g.ml^{-1} - 0.03 g.ml^{-1} could increase the activities of protective enzymes (SOD and CAT) and the non-protective enzyme (PAL and PPO) of tomato as compared to the control. These results are in agreement with Han *et al.*, (2013) that the decomposed garlic stalk at lower concentrations significantly increased the growth of lettuce and promoted physiological indices and activities of plant enzymes in lettuce leaf.

Induction of POD activity in plants occurs in response to numerous biotic and abiotic stimuli, including exposure

to pathogens, chemical oxidizing agents, and red light (Casal *et al.*, 1994). Peroxidase plays role in auxin catabolism, production and breakdown of hydrogen peroxide. The anti-oxidant capabilities of POD make it as an important factor in the integrated defense response of plants to a variety of stresses (Cipollini, 1998). In present study the level of POD activity increased in the intercropped treatments due to positive effect of the allelochemicals and enhanced the defense ability of the pepper plant. Plants release the allelochemicals as mixture rather than a single compound (Makoi and Ndakidemi, 2007). The effects of individual constituents are often different from mixtures, in which the synergic and antagonistic effects occur (Rizvi *et al.*, 1999). These results are in agreement with Cui *et al.*, (2011), who reported that activities of SOD, POD and CAT enzymes in turnip were higher when it was treated with root exudates of 2 and 3 years old walnut trees and they further reported that increase in POD activity in response to high level of free oxygen radicals was due to alleochemicals. Similar results were reported by Jing *et al.*, (2003) that allelopathic agents increased the activities of POD and SOD in cucumber, two important enzymes for the detoxification of overproduced active oxygen species (AOS). AOS brings about peroxidation of membrane lipids, which leads to membrane damage. These results are supported by Naseer & Bano (2013) that significant increases were rerecorded in the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in response to application of fresh as well as oven dried leaf extracts prepared from drought stressed maize plants.

Plant enzymes are involved in defense reactions against plant pathogens and it include oxidative enzymes such as polyphenol oxidase (PPO), which catalyze the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure. Such enzymes have been correlated with defense against pathogens in several plants including tobacco, tomato, cucumber and rice. PPO activity is significantly decreased in all the intercropped treatments as compared to the control and it might be due to allelochemicals released by garlic which caused oxidative damage through an increase in reactive oxygen species (ROS) and modification of PPO. These results are in line with the findings drawn by Lixuan *et al.*, (2007), that the activities of defense enzymes such as polyphenol oxidase (PPO) in leaves and roots of watermelons in the intercropping system were significantly lower than those in the mono-cropped system. They further reported that enzyme inhibition decreased dehydrogenase activity and increased the level of reactive oxygen species (ROS).

Conclusion

It is of great importance to take advantage of intercropping in the efforts for maintaining biodiversity and sustainable agricultural development. During this study pepper and garlic based intercropping system improved physiological properties of pepper. It can be concluded from this study that intercropping pepper with normal garlic had good impact on chlorophyll contents, photosynthetic rate and antioxidant enzymes as compared to other treatments.

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