COLOCASIA BASED CROPPING SYSTEMS AFFECTS THE ANTIOXIDANT PROPERTIES AND PRODUCTIVITY OF COLOCASIA [COLOCASIA ESCULENTA (L.) SCHOTT] TUBER

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ABSTRACT

Four colocasia based cropping system viz Colocasia-Onion-Frenchbean (C-O-F), Colocasia-Frenchbean-Potato (C-F-P), Colocasia-Cabbage-Frenchbean (C-Ca-F), Colocasia-Coriander-Tomato (C-Co-T) were taken to find out the effect of different crops under colocasia based cropping system on the antioxidant activities (AA) and productivity of colocasia tuber. The highest concentrations of phenolics (0.966 + 0.009 mg gallic acid equivalent/100 mg fresh weight) and condensed tannins (0.022 + 0.001 mg catechins equivalent/100 mg fresh weight) were found in 'C-O-F', while the anthocyanin (4.29 + 0.04 mg/100 mg fresh weight) were found highest for 'C-Co-T' cropping system. Colocasia samples from 'C-O-F' cropping system showed highest DPPH and ABTS radical scavenging activities, while reducing power activity was found highest in the colocasia samples from 'C-Co-T' cropping system. Principal component analysis (PCA) showed strong correlation between phenolics, tannins and AA, while anthocyanin was found positively correlated with reducing power. Results of the finding provides evidence that the colocasia samples for 'C-O-F' cropping system showed higher antioxidant activities than samples from other cropping system in most of the determinations, while the productivity in terms of colocasia equivalent yield (52.38 ton/ha) was recorded highest in the 'C-O-F' cropping system.

INTRODUCTION

Antioxidants may be defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate in a chain reaction (Halliwell and Whitemann, 2004; Leong and Shui, 2002) Antioxidants have become a popular research topic because they cannot be generated by the human body and hence have to be consumed in the diet. A major benefit from the diets rich in fruit and vegetables may be increased consumption of antioxidant vitamins such as ascorbate (vitamin C) and tocopherol (vitamin E), vitamin-like compounds (glutathione), and pigments such as phenolics and carotenoids (Moyer et al., 2002). They act as major cellular redox buffers that can effectively quench reactive oxygen species (ROS) by donating one or more electrons to ROS. Natural phytochemicals content in fruits and vegetables were greatly affected by growing condition and environment (Jeannelle and Rui, 2004).

Colocasia esculenta (L.) Schott. belongs to family araceae, commonly known as Taro (English). In India, Colocasia is traditionally used as an abortifacient, to treat tuberculous ulcers, pulmonary congestion, crippled extremities, fungal abscesses in animals, and as an anthelmintic (Singh et al., 2011; Kubde et al., 2010; Tattiyaakul et al., 2006). Recent pharmacological studies reveals that leaves of C. esculenta reported to had antibacterial, antifungal (Singh et al., 2011), anthelmintic (Kubde et al., 2010), anti-inflammatory (Biren et al., 2007) and anti diabetic Kumawat et al., 2010) activities. Moreover, α-amylase inhibitory activity (McEwan et al., 2010), antidiabetic (Prakasam et al., 2003), in vivo antiperoxidative and antioxidant activity (Prakasam et al., 2005) were also attributed to tuber or root of C. esculenta.

Depending on the resources and technology available, different types of cropping systems are adopted by farmers. It may be due different sizes of fields, different types of soil, and may be on a slope or on flat land. But it is well accepted that cropping systems are the intensification of cropping in time and space dimensions (Mandal et al., 2014). Different phytochemicals in plant system acts synergistically to increase their antioxidant effects. Along with high antioxidative properties and yield of colocasia tuber as affected by different colocasia based cropping systems, the present study has been undertaken with the novel colocasia-based cropping systems. The objective of this study was to assess and compare the variations in antioxidative capacity and economic yield of colocasia in novel colocasia-based cropping systems in Indian sub-Himalaya

MATERIALS AND METHODS

Experimental details

The field experiments were carried out at the research farm of the Vivekananda Parvatiya Krishi Anusandhan Sansthan, Hwailbagh, Almora, India during year 2007-10. The site was located at 29°36’ N latitude and 79°40’ E longitude at an

Received on : 21.08.2014
Accepted on : 07.01.2015

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elevation of 1250 m above mean sea level. The three cropping seasons at this site include a rainy or kharif season from June to October, a winter or rabi season from November to February, and a summer or dry season from March to May. The soil was clay loam. The four cropping systems were: Colocasia-Onion-Frenchbean (C-O-F), Colocasia-Frenchbean-Potato (C-F-P), Colocasia-Cabbage-Frenchbean (C-Ca-F), Colocasia-Coriander-Tomato (C-Co-T). These systems were arranged in a randomized block design (RBD) with three replications. The net plot size was 13.5m². The crop was raised on natural soil fertility and the nutritional requirements of the crop were met through application of mineral fertilizers and farmyard manure (FYM). At the end of third year colocasia samples were collected and antioxidant properties and economic yield were recorded.

Extraction for phenolic compounds, condensed tannins and total anthocyanins

Five (± 0.2) grams, of fresh edible part of colocasia tuber was homogenized in 25mL of extraction solvent (400mL of acetone/400 mL of methanol/200 mL of water/10 mL of acetic acid) as described by Rababah et al. (2005) with some modification. The homogenate was transferred into a 50 ml Oak Ridge centrifuge tube and incubated in a water bath at 60ºN for 1h followed by a 3 min sonication. Sonicated samples were clarified by centrifugation at 13,000 rpm for 15 min at 4ºN, then filtered with Whatman filter paper no 1 and diluted to a final volume of 50 mL. Samples were stored in a 4ºN until time of analysis.

Determination of total phenolics (TP)

Total phenolics (TP) content was determined spectrophotometrically by the Folin-Ciocalteau method (Singleton and Rossi, 1965). Extracts (200 μL) or gallic acid standard solutions were mixed with 2.6 mL of double distilled water. Generation of a standard curve was achieved by constructing five different concentrations of gallic acid (20, 40, 60, 80 and 100 mg/L). A blank was prepared using double-distilled water instead of a sample. Subsequently, 200 μL of Folin-Ciocalteau Reagent (FCR-1:5 dilution with double distilled water) were mixed with the sample, standard or blank. The reaction mixture was allowed to stand at room temperature for 6 min to permit the FCR reagent to react completely with oxidizable substrates or phenolates. Following incubation, 2.0 mL of 7% Na2CO3 solution were added to each mixture and allowed to stand at room temperature for 90 min. The absorbance was measured at 750 nm. Results are expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight based on three replications per sample or standard.

Determination of condensed tannins (CT)

Condensed tannins were estimated using the method of Sun et al. (1998) with some modifications. To the freshly prepared extract (0.1 mL), 0.9 mL methanol, 2.5 mL of 1 % vanillin reagent and 2.5 mL of 9M HCl was added. The solution was mixed thoroughly and absorbance at 500 nm was recorded after 20 min of incubation at 30ºC. Condensed tannins content was calculated from the standard calibration curve based on catechins.

Determination of total anthocyanins (Anthro)

Total anthocyanins analysis was performed following the method of Giusti and Wrolstad (2005). Briefly, colocasia extract (100 μL) was diluted with two different solutions (900 μL each): 0.025 M potassium chloride bufer, pH = 1.0 and 0.4 M sodium acetate bufer, pH = 4.5. The absorbance was measured at maximum (510–520 nm) and, 700 nm against a blank cell filled with distilled water. The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated: A = (A Vis-max - A700 nm) pH 1.0 - (A Vis-max - A700 nm) pH 4.5

The monomeric anthocyanin pigment concentration was calculated using the following equation: Monomeric anthocyanin pigment (mg/L) = (A x MW x DF x 1000)/(ε x λ)

where MW = 449.2 and £ = 26900 are, respectively, the molecular weight and molar absorptivity of cyanidin 3-O-glucoside that was used as a standard and was one of the major anthocyanins; DF is the dilution factor and λ is the path length (cm). The total monomeric anthocyanins were reported on the basis of mg/100 g FW colocasia tuber.

Extraction for antioxidant activity measurements

Methanolic extract of colocasia samples were taken to measure antioxidant activities. Tubers were weighed, peeled, fractionated into little pieces and dried at 40ºC in a hot air oven to constant weight (Eleazu et al., 2011). The dried samples were ground to fine powder by using an electric grinder. Two (2.0) grams blended samples were extracted by semiautomated soxlet apparatus (pelican, socsplus, 2AS, Chennai) in methanol at 100ºC for 1h and 90% methanol was recovered during recovery phase at 130ºC for 30 min. The methanolic extract of each were then evaporated at 80ºC in the dryness, redissolved in methanol to a concentration of 10 mg/ml and stored at 4ºC for further use. The all assays were carried out in triplicate and the results are expressed as mean values ± standard error.

Determination of scavenging effects on DPPH radicals

The DPPH assay was done by measuring the decrease in absorbance of methanolic DPPH solution at 515 nm in the presence of the extract (Brand-Williams et al., 1995). The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20ºC until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.17 ± 0.02 units at 515 nm. Methanolic extract (150 μL) of different concentrations (0.5, 1.0 1.5 and 2 mg mL-1) were allowed to react with 2850 μL of the working DPPH solution for 24 h in the dark. Then the absorbance was measured at 515 nm. Butylated hydroxytoluene (BHT) was employed as a reference and the radical scavenging activity was calculated as the percentage of DPPH discoloration using the equation: DPPH radical scavenging (%) = [(A Vis-max - A sample)/A Vis-max] x 100, Where A sample is the absorbance of the solution when the extract/reference has been added at a particular level, and A Vis-max is the absorbance of the DPPH solution without extract added.

Determination of scavenging effect on ABTS radicals

The ABTS assay was done by measuring the decrease in absorbance of methanolic ABTS solution at 745 nm in the presence of the extract (Arnao et al., 2001). The stock solutions included 7.0mM ABTS solution and 2.3mM Ammonium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and...
allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL of ABTS solution with 3 mL methanol to obtain an absorbance of 0.9 ± 0.02 units at 745 nm. Methanolic extract (200 μL) of different concentrations (0.5, 1.0, 1.5 and 2 mg/mL) were allowed to react with 2000 μL of the ABTS solution for 30 min in a dark condition. Then the absorbance was taken at 745 nm by using the spectrophotometer. BHT was employed as a reference.

Reducing power assay

The reducing power assay was determined following the method of Huda Fujan et al. (2009). Various concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL) of methanolic extracts (200 μL) were taken and volume made up to 1 mL by adding distilled water, in these added 2.5 mL of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. Afterward 2.5 mL of 10% trichloroacetic acid (w/v) were added; the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of a solution containing 0.1% of ferric chloride and the absorbance was measured at 700 nm by spectrophotometer. Higher absorbance indicates higher reducing power. The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance at 700 nm against extract concentration.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g C3H5NaO2.3H2O and 16 mL C2H4O2), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM FeCl3·6H2O solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL FeCl3·6H2O solution and then warmed at 37ºC before using. Methanolic extracts (150 μL) were allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. Readings of the colored product were taken at 593 nm. The FRAP value was determined by plotting in a standard curve produced by the addition of ferrous sulphate (Merck, Darmstadt, Germany) to the FRAP reagent.

Determination of total antioxidant activity

The total antioxidant activity of the methanolic extract of both the sample was measured by spectrophotometrically using a phosphomolybdenum method (Prieto et al., 1999), based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of specific green phosphate / Mo (V) compounds. Sample extract (0.3 mL) of different concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL) were combined with 2.7 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was capped and incubated in a boiling water bath at 95ºC for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 750 nm. The total antioxidant activity was expressed as equivalents of trolox (μM/g of extract).

Yield measurements and Colocasia equivalent yield (CEY)

Yields of main and by-products of each crop under various cropping systems were measured by harvesting 13.5 m² area in each plot at physiological maturity of respective crops. The economic part of individual crops was separated manually and harvested. All crops were cut at about 15 cm from the surface, except the potato and coriander (Biswa et al., 2006) Colocasia equivalent yield (CEY) was calculated to compare performance of several cropping systems by converting the economic yield of each crop into equivalent colocasia yield on a price basis, using the formula: CEY (of crop x) = Yx (Px/Pc)

Where, Yx is the yield of crop x (tons economic harvest product ha⁻¹), Px is the price of crop x, and Pc is the price of colocasia.

Statistical analysis

The statistical analyses were performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc.,
followed the order 'C-O-F' > 'C-Co-T' > 'C-F-P' > 'C-Ca-F' (Fig. 6).

The productivity in terms of colocasia equivalent yield (CEY) of all four cropping systems was determined using the SAS JMP 9.0 version software. Multivariate analysis was carried out using the using the SAS JMP 9.0 version software.

RESULTS AND DISCUSSION

Total phenolics (TP), Condensed Tannins (CT) and Total Anthocyanin

The amount of total polyphenol (TP), condensed tannins (CT) and total anthocyanin in colocasia among all cropping system were tabulated in Table 1. The TP was determined from the regression equation of the calibration curve obtained from gallic acid (y = 0.0033x, r > 0.99). The TP was found maximum (0.966 + 0.009 mg GAE/100 mg FW) in colocasia samples from 'C-O-F' cropping system and found minimum (0.85 + 0.029 mg GAE/100 mg FW) in colocasia samples from 'C-Co-T' cropping system which was the non-significantly at par with the 'C-F-P' and 'C-Ca-F' cropping system (Table 1). The values of total polyphenols are comparable to those of the result found by Basu et al. (2012) from Colocasia esculenta. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Smirnoff and Cumbes, 1989; Komali, 1999). The total CT content from the crops extract was assayed by vanillin-HCl colorimetric assay as described in materials and methods, determined from regression equation of calibration curve (y = 0.0022 x + 0.013536; r = 0.997) and expressed in catechins equivalents.

The result showed that The CT was found maximum (0.022 + 0.001 mg CE/100 mg FW) in colocasia samples from 'C-O-F' cropping system followed by the sample from 'C-Co-T' cropping system (0.016 + 0.001 CE/100 mg FW). Content of condensed tannins was found non-significantly at par between 'C-Ca-F' and 'C-F-P' cropping system (Table 1). Condensed tannins are very important plant constituents because of having active hydroxide ions (OH⁻) and show antioxidant activity (Eric et al., 2011).

The table 1 showed that the highest levels of total anthocyanin (4.29 + 0.04 mg/100 mg FW) was found in colocasia samples from 'C-Co-T' cropping system, followed by 'C-Ca-F' and the lowest (4.04 + 0.09 mg/100 mg FW) in 'C-O-F' cropping system. The result were comparable with the as reported by Prajapati et al., 2011.

Free radical scavenging Activities

The abilities for each concentration of the extract samples to scavenge DPPH and ABTS radicals are shown in figure 1 and 2 respectively. Scavenging effect (% inhibition) of the methanolic extracts from the sample of all the cropping systems on DPPH radicals increased with increase in concentration. At 0.5 to 2.0 mg/mL, the scavenging activities of methanolic extracts of colocasia samples from 'C-Co-T', 'C-O-F', 'C-F-P', 'C-Ca-F' cropping system on DPPH radical ranged from 21.95 to 41.09, 20.73 to 52.59, 11.63 to 39.96, and 14.46 to 40.46 %, respectively (Fig. 1). However, BHT at same concentration showed excellent DPPH scavenging activities (74.28 to 95.89 % inhibition). Colocasia from 'C-O-F' cropping system showed highest, while 'C-F-P' cropping system showed lowest DPPH scavenging activities.

Table 1: Total polyphenols, condensed tannins and total anthocyanin content in colocasia samples from four different colocasia based cropping system

<table>
<thead>
<tr>
<th>Cropping system</th>
<th>Total Phenolics (mg GAE/100 mg FW)</th>
<th>Condensed Tannins (mg CE/100 mg FW)</th>
<th>Total anthocyanin (mg/100 mg FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Co-T</td>
<td>0.85 ± 0.029 ±</td>
<td>0.016 ± 0.001 ±</td>
<td>4.29 ± 0.04 ±</td>
</tr>
<tr>
<td>C-O-F</td>
<td>0.966 ± 0.009 ±</td>
<td>0.022 ± 0.001 ±</td>
<td>4.04 ± 0.09 ±</td>
</tr>
<tr>
<td>C-F-P</td>
<td>0.868 ± 0.011 ±</td>
<td>0.010 ± 0.001 ±</td>
<td>4.09 ± 0.06 ±</td>
</tr>
<tr>
<td>C-Ca-F</td>
<td>0.864 ± 0.014 ±</td>
<td>0.011 ± 0.001 ±</td>
<td>3.95 ± 0.03 ±</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significant difference using LSD (p < 0.05); Values are the mean of three determinations; C: Colocasia, O: Onion, F: Frenchbean, P: Potato, Ca: Cabbage, Co: Coriander; GAE: Gallic acid Equivalent, CE: Catechins Equivalent, FW: Fresh Weight

Table 2: EC₅₀ values (mg/mL) of colocasia extract from four different Cropping systems in the antioxidant activity evaluation assays

<table>
<thead>
<tr>
<th>Cropping system</th>
<th>EC₅₀ values of each free radical scavenging assay (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH²</td>
</tr>
<tr>
<td>C-Co-T</td>
<td>2.630</td>
</tr>
<tr>
<td>C-O-F</td>
<td>1.390</td>
</tr>
<tr>
<td>C-F-P</td>
<td>2.890</td>
</tr>
<tr>
<td>C-Ca-F</td>
<td>2.680</td>
</tr>
<tr>
<td>BHT</td>
<td>0.320</td>
</tr>
</tbody>
</table>

EC₅₀ (mg/mL): effective concentration at which 50% of DPPH radicals are scavenged.

² EC₅₀ (mg/mL): effective concentration at which 50% of ABTS radicals are scavenged.

³ EC₅₀ (mg/mL): effective concentration at which 50% of RPA radicals are scavenged.

C: Colocasia, O: Onion, F: Frenchbean, P: Potato, Ca: Cabbage, Co: Coriander; The productivity in terms of colocasia equivalent yield (CEY) of all four cropping systems was followed the order 'C-O-F' > 'C-Co-T' > 'C-F-P' > 'C-Ca-F' (Fig. 6).
ABTS it was BHT > 'C-O-F' > 'C-F-P' > 'C-Co-T' > 'C-Ca-F'. Reducing power serves as a significant indicator of potential as antioxidant. Colocasia samples from four different cropping systems were used to evaluate the reducing power at four different extract concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml). Reducing power significantly increased with the increase in extract concentration in a dose-dependent manner (P < 0.05). Colocasia sample from 'C-Co-T' showed highest reducing power followed by 'C-O-F' and lowest was by 'C-F-P' cropping system at all the four concentrations (Fig. 3).

It was reported that reducing power activities are associated with the presence of reducing agents, which shows antioxidant action by donating a hydrogen atom and breaking the free radical chain (Mathew and Abraham, 2006).

**Ferric Reducing Antioxidant Power (FRAP)**

FRAP assay is a colorimetric method based on the reduction of a ferric tripyridyltriazine (TPTZ) complex to its ferrous form. This reduction originates an intense blue complex with an absorption maximum at 593 nm (Benzie and Strain, 1996).

The antioxidant capacity of colocasia samples from all four cropping system systems were evaluated and expressed as FRAP value, are shown in Fig. 4.

Colocasia samples from ‘C-O-F’ showed highest FRAP value and FRAP value significantly increased with the increase in extract concentration in a dose-dependent manner.

**Total Antioxidant Activity**

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, á-tocopherol, and carotenoids (Sarikurkcu et al., 2008). Total antioxidant activity significantly increased with the increase in extract concentration in a dose-dependent manner (P < 0.05). At all four extract concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml), colocasia samples from ‘C-O-F’ showed highest total antioxidant activity (41.31, 54.57, 95.09, 115.04 μM Trolox equivalent) respectively, while minimum activity was recorded in ‘C-F-P’ cropping system at most of the concentrations (Figure 5).

The total antioxidant activity of plant seems to be due to the...
The highest radical scavenging activities and total antioxidant correlation also showed that cropping system ‘C-O-F’ showed catechins. The principal component analysis (PCA) and their relationship in different cropping system (Parihar et al., 2010). Several studies reported (Goffman and Bergman, 2004; Itani et al., Tatemoto, H., Okamoto, M., Fujii, K. and Muto, N. 2002). It suggests that total polyphenols (TP) and condensed tannin (CT) were positive correlated with ABTS, DPPH and total antioxidant (TA) activities, while total anthocyanin (Antho) was positively correlated with reducing power (RP). Previously several studies reported (Goffman and Bergman, 2004; Itani et al., 2002; Pal et al., 2013) that antioxidant activity have positive correlation with the total phenol content and is especially associated with the content of tannic acid and catechins. The principal component analysis (PCA) and their correlation also showed that cropping system ‘C-O-F’ showed highest radical scavenging activities and total antioxidant activities.

Principal Component Analysis (PCA)

Principal component analysis clearly indicates correlation between various antioxidant activities and related parameters and their relationship in different cropping system (Parhar et al., 2013). The principal component analysis (PCA) and their correlation are shown in Figure 6. The first principal component represents 57.5 per cent of variability, while the second principal component represents 34.4 per cent of variability among the data. Almost all parameters were occupied on the right side of the biplot.

This suggests that total polyphenols (TP) and condensed tannin (CT) were positive correlated with ABTS, DPPH and total antioxidant (TA) activities, while total anthocyanin (Antho) was positively correlated with reducing power (RP). Previously several studies reported (Goffman and Bergman, 2004; Itani et al., 2002; Pal et al., 2013) that antioxidant activity have positive correlation with the total phenol content and is especially associated with the content of tannic acid and catechins. The principal component analysis (PCA) and their correlation also showed that cropping system ‘C-O-F’ showed highest radical scavenging activities and total antioxidant activities.

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