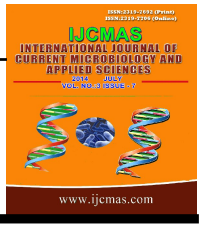
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 7 (2014) pp. 1018-1027**
http://www.ijcmas.com



Original Research Article

Impact of AM fungi on biochemical changes in potato plants

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A B S T R A C T

This research article aimed at determining the impact of AM fungi on changes in key biochemical components of potato plants. Potato tubers were grown in pots with mycorrhiza and without mycorrhiza. After 60 and 90 days, leaves and tubers were collected for biochemical studies. Chlorophyll content, proteins, total carbohydrates, ascorbic acid, starch, peroxidase, polyphenol oxidase, acid and alkaline phosphatase were assessed during our study. Results obtained clearly indicated increase in chlorophyll, protein, total carbohydrates, ascorbic acid, starch, peroxidase and polyphenol oxidase levels when plants were grown with mycorrhiza. Alkaline and acid phosphatase analysis showed lowered levels with mycorrhiza thereby confirming their role in achieving symbiosis. Collaborated results confirmed the positive effect on biochemical and nutritive capacity of mycorrhizal plants

Keywords

AM fungi, Biochemical contents, Potato

Introduction

Mycorrhizal occurrence in terrestrial plants has been long known and it is now a known fact that AM fungi positively influence plant growth. The term 'Mycorhiza' was coined by Frank (1885) for the non pathogenic, symbiotic association between roots and fungi. These are members of the fungal group that mutualistically associate themselves with plant roots. The relation is mutualistic due to the increased mineral availability to plants by activity of AM fungi and a complementary nutritional and habitat security offered by the plant member.

Asai (1943) used sterilized and non-sterilized (mycorrhizal) soil and obtained results that indicated faster growth of mycorrhizal plants than the non-mycorrhizal plants. Moving forward, many such studies indicated a positive growth influence in mycorrhizae associated plants than otherwise. Plant growth is actually an effect of physiological activities, invisible to the unaided eye. It requires specialized techniques that assist us in determining and comparing the concentrations and activities of biochemical contents.

In our study, we tested the presence and quantified some key biochemical parameters in potato plant parts grown with mycorrhiza and without mycorrhiza.

Materials and Methods

Potato plants were grown in two set of pots, marked by the presence and absence of AM fungi. Plant parts were collected for assessment of nine different biochemical components after 60 and 90 days of plant growth. The parameters studied include chlorophyll, proteins, total carbohydrates, ascorbic acid, starch, peroxidase, polyphenol oxidase, acid phosphatase and alkaline phosphatase. Chlorophyll a, b and total chlorophyll content was estimated using Arnon's method (1949). In this method, fresh potato leaves from mycorrhizal and non-mycorrhizal plants were collected and used for the process. The absorbance of the two sets was determined at 645 nm and 663 nm using UV-visible spectrophotometer. The protein content of mycorrhizal and non-mycorrhizal potatoes was estimated using Lowry *et al.* (1951) method. Potato tubers were used for protein estimation and the same were treated with the four main reagents of this method. Absorbance readings were taken at 660 nm.

The total carbohydrates content was estimated using the anthrone method proposed by Hedge and Hofreiter (1962). For the estimation purpose, freshly weighed mycorrhizal and non-mycorrhizal potato tubers were used and readings for absorbance were taken at 630 nm.

The total amount of peroxidase enzyme in potato leaves was estimated from mycorrhizal and non-mycorrhizal plants using method proposed by Putter (1974) and Malik and Singh (1980). Potato plant leaves were used and absorbance was read at 436nm.

The total amount of polyphenol oxidase enzyme in potato leaves was calculated from mycorrhizal and non-mycorrhizal plants using the method proposed by Esterbaner *et al* (1977). Absorbance readings were taken at 495 nm for calculation of enzyme concentration.

The total amount of alkaline phosphatase was calculated from the leaves of mycorrhizal and non-mycorrhizal potato plants using the method proposed by Lowry (1954). Absorbance readings were taken at 405 nm. The total amount of acid phosphatase was calculated from leaves of potato using mycorrhizal and non-mycorrhizal plants by the method proposed by Lowry (1954). Absorbance readings were taken at 405 nm using a UV Spectrophotometer.

Ascorbic acid or vitamin C content of mycorrhizal and non-mycorrhizal potato tubers was estimated using the volumetric method (Sadashivam and Balasubramanian, 1987; Harris and Ray, 1935).

The total amount of starch present in potato tubers was estimated using the anthrone method proposed by Hedge and Hofreiter (1962). Absorbance readings were taken at 630 nm.

Results and Discussion

The chlorophyll content of potato plant leaves was estimated with and without AM fungi and the same was analyzed for its standard deviation. A total of two estimations were made to compare the gradual changes in the chlorophyll content of potato plants.

Plants grown in the absence of AM fungi, when examined for the amount of chlorophyll a showed 1.540±0.103 mg/g on 60^{th} day, while mycorrhizal plants reported 2.173±0.0770 mg/g. On the 90^{th} day, the chlorophyll a content recorded was

1.439±0.122 mg/g in non-mycorrhizal plants and 2.077±0.1160 mg/g in mycorrhizal plants.

content was 0.125±0.080 mg/g in the non-mycorrhizal plants and 0.189±0.093 mg/g in mycorrhizal plants after 60 days of plantation. Maximum concentration of chlorophyll b was observed in potato leaves that were collected on the 90th day that was 0.142±0.080 mg/g in non-mycorrhizal plants and 0.183±0.097 mg/g in mycorrhizal plants.

The amount of total chlorophylls when measured at 60th day was observed as 1.652±0.108 mg/g in non-mycorrhizal plants and 2.205±0.106 mg/g in mycorrhizal plants. Maximum amount of total chlorophylls content was observed in the leaves collected at 90th day and was 1.565±0.073 mg/g in non-mycorrhizal plants, whereas it was 2.162±0.083 mg/g in mycorrhizal plants.

Estimation of Protein content

The amount of proteins present in potato tubers grown in the absence of AM fungi was calculated 60 days after plantation; the protein content was seen to be 1.60±0.051 g/100g in non-mycorrhizal plants while mycorrhizal plants depicted 1.91±0.035 g/100g sample. Maximum amount of protein concentration however was observed on 90th day that was 1.56±0.052 g/100g in non-mycorrhizal plants and 1.89±0.040 g/100g in mycorrhizal plants. Mycorrhizal plants possessed greater quantity of proteins in comparison to control plants.

Total carbohydrate estimation

The amount of total carbohydrates was measured after 60 days of plantation and the amount present in non-mycorrhizal plants was 16.31±0.284 mg/g and 17.25±0.250 mg/g in mycorrhizal plants. Maximum amount was seen when plants were examined on 90th day. In non-mycorrhizal plants the amount of total carbohydrates was 16.55±0.390 mg/g in control plants and was 17.46±0.256 mg/g in mycorrhizal plants.

Estimation of Polyphenol oxidase and peroxidase from potato tubers

The amount of peroxidase units was estimated on 60th day and was 1.143±0.04041 units/litre in non-mycorrhizal plants. Mycorrhizal plants on the other hand depicted higher value that was 3.183±0.0763 units/litre. This amount was seen to considerably increase to maximum concentration after 30 days to 1.243±0.04041 units/litre in non-mycorrhizal plants and 4.416±0.0763 units/litre in mycorrhizal plants. Maximum level of peroxidase was observed at 90 days in mycorrhizal plants.

The amount of polyphenol oxidase was also estimated on 60th day and plants grown in the absence of AM fungi depicted 0.0064±0.00045 consumption of 1µmole dihydricphenol/min, while plants growing in the presence of AM fungi depicted 0.0135±0.0005 consumption of 1µmole dihydricphenol/min. 90 days after plantation, the non-mycorrhizal plants depicted 0.0074±0.00036 and mycorrhizal plants 0.0153±0.0010 of consumption of 1µmole dihydricphenol/min but maximum levels were reported after 90 days in mycorrhizal plants.

Collectively, the results obtained for the enzyme polyphenol oxidase and peroxidase depicted that the amount of these enzymes was greater in plants that were colonized with AM fungi than the plants that were grown in the absence of AM fungi.

Estimation of alkaline and acid phosphatase from potato tubers

Acid phosphatase levels depicted a unique pattern of presence within potato plants. The amount of acid phosphatase in non-mycorrhizal plants was recorded 0.216±0.0076 mM pnp/min/mg protein after 60 days of plantation, and in case of mycorrhizal plants it was reported 0.188±0.0080 mM pnp/min/mg protein. The amount of acid phosphatase in 90 days old plants recorded 0.342±0.0075 and 0.271±0.0076 mM pnp/min/mg protein without and with mycorrhiza respectively. The concentration of enzyme was higher in non-mycorrhizal plants than in mycorrhizal plants at 90 days. Hence, 90 days old non-mycorrhizal plants possessed highest concentrations of the enzyme.

The amount of alkaline phosphatase in 60 days old non-mycorrhizal plants was observed as 0.187±0.0025 mM pnp/min/mg protein. Mycorrhizal plants on the other hand contained 0.163±0.0125 mM pnp/min/mg protein. This amount was observed to increase when the next sampling was done. Non-mycorrhizal plants that were 90 days old contained 0.284±0.0040 mM pnp/min/mg protein and mycorrhizal plants contained 0.233±0.0076 mM pnp/min/mg protein. Maximum amount of the enzyme was observed in 90 days old non-mycorrhizal plants. The amount of alkaline phosphatase was recorded less in the plants rose with AM fungi than the plants raised without AM fungi. This result was concomitant in each phase of analysis that is 60 and 90 days.

Ascorbic acid estimation from potato tubers

The amount of ascorbic acid was calculated for non-mycorrhizal plants on 60th day and was found 18.083±0.5204 mg/100g. It was observed 19.366±0.1258 mg/100g in mycorrhizal plants. The amount of ascorbic acid in non-mycorrhizal plants was increased and was reported to be 17.216±0.7973 mg/100g after 90 days plantation and that in mycorrhizal plants was recorded 18.633±0.0763 mg/100g. Maximum amount of ascorbic acid was observed in plants that were 90 days old. Mycorrhizal plants possessed greater amount of ascorbic acid than control plants.

Estimation of starch from potato tubers

After 60 days, the samples were collected and same were analyzed for the amount of starch. Non-mycorrhizal plants showed 13.400±0.1322 g/100g starch and mycorrhizal plants contained 14.633±0.1040 g/100g of starch. The values increased when plant was 90 days old up to 13.866±0.1040 g/100g in non-mycorrhizal plants and 15.300±0.0500 g/100g in mycorrhizal plants. Maximum starch content was observed in potatoes that were 90 days old. Mycorrhizal plants contained more starch than non-mycorrhizal plants.

During our study, key biochemical parameters were studied for understanding the changes plants experience in terms of its biochemical composition when they are colonized with AM fungi. Mentioned below are the explanations and understandings we confer after analysis of the results obtained.
Chlorophyll contents of leaves upon estimation displayed higher values of chlorophyll a, chlorophyll b and total chlorophylls in mycorrhizal plants as compared to control plants. Mycorrhizal plants depicted 1.38 times higher amount of total chlorophylls than control plants that indicates a greater scope for mycorrhizal plants to produce food by photosynthesis (Chart 1).

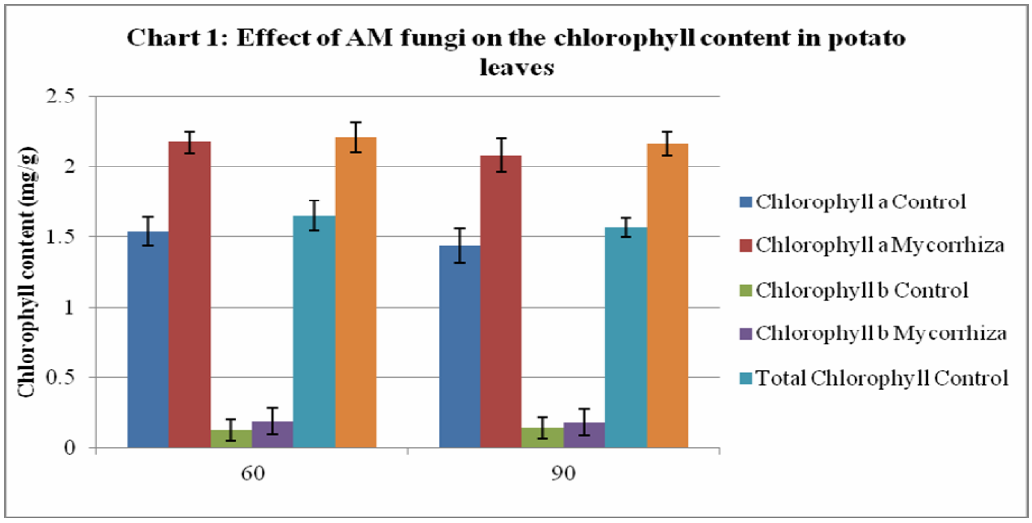
Chart 1: Effect of AM fungi on the chlorophyll content in potato leaves
Chlorophyll content (mg/g)
2.5
2
1.5
1
0.5
0
60
90
Chlorophyll a Control
Chlorophyll a Mycorrhiza
Chlorophyll b Control
Chlorophyll b Mycorrhiza
Total Chlorophyll Control

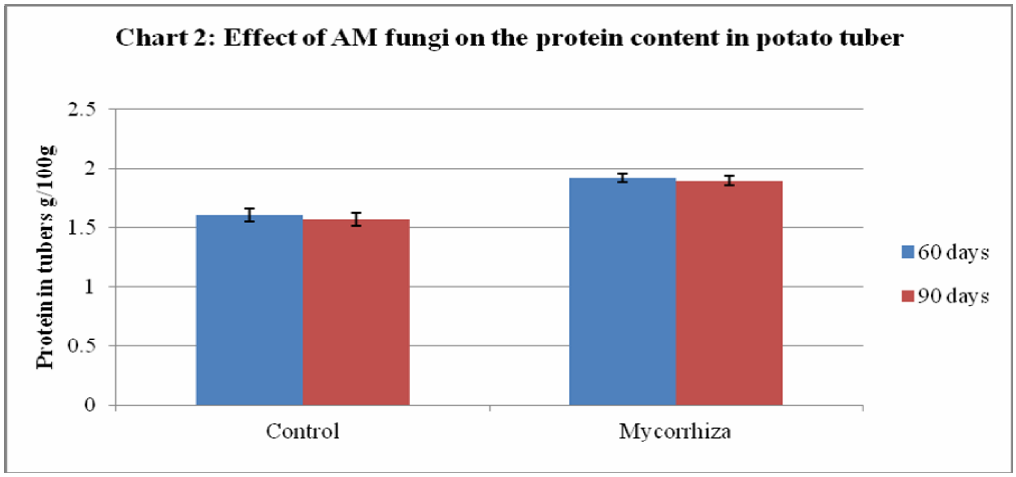
Chart 2: Effect of AM fungi on the protein content in potato tuber
Protein in tubers g/100g
2.5
2
1.5
1
0.5
0
Control
Mycorrhiza
60 days
90 days

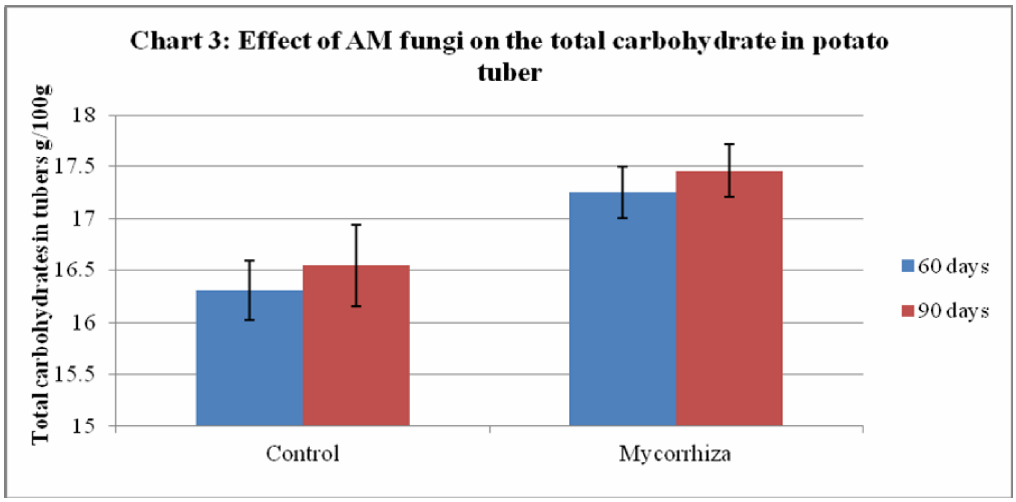
Chart 3: Effect of AM fungi on the total carbohydrate in potato tuber
Total carbohydrates in tubers g/100g
18
17.5
17
16.5
16
15.5
15
Control
Mycorrhiza
60 days
90 days

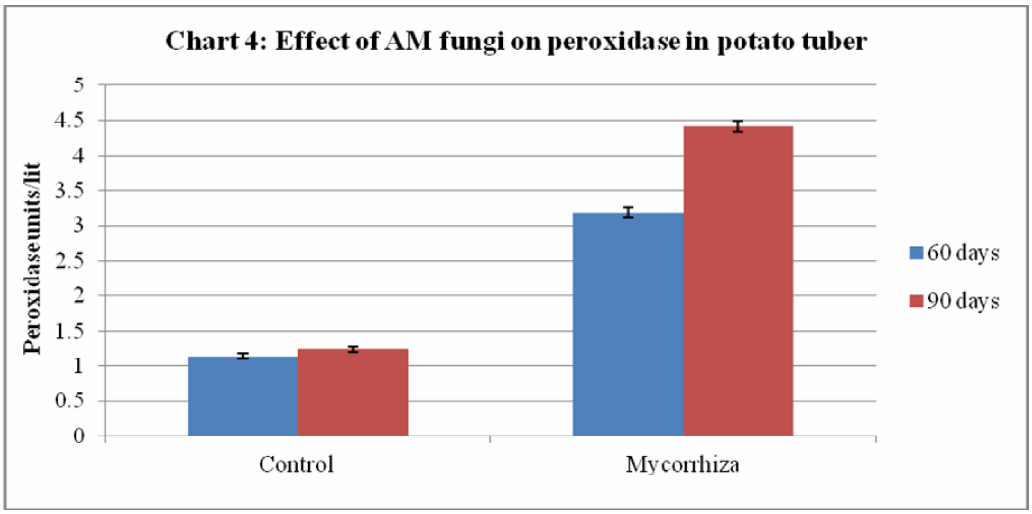
Chart 4: Effect of AM fungi on peroxidase in potato tuber
Peroxidase units/lit
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
Mycorrhiza
60 days
90 days

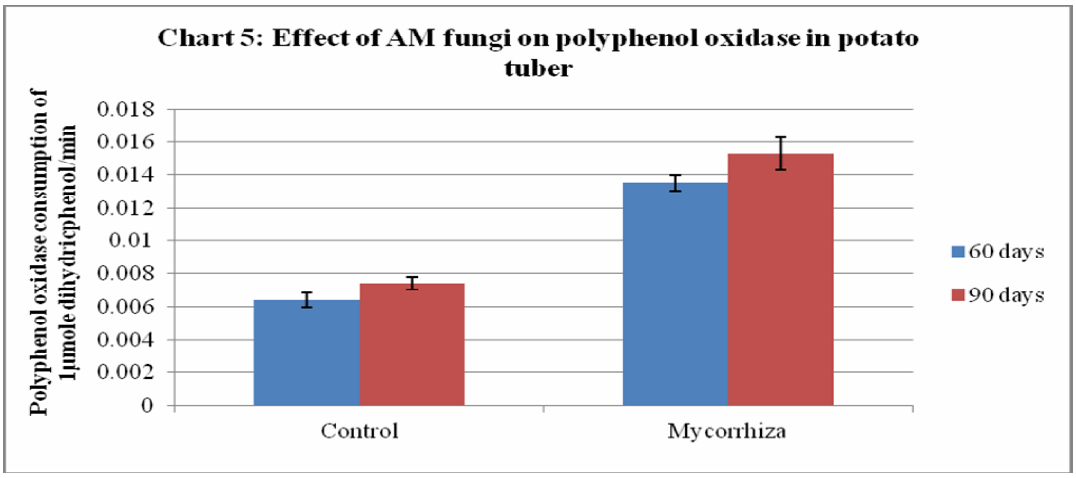
Chart 5: Effect of AM fungi on polyphenol oxidase in potato tuber
Polyphenol oxidase consumption of 1µmole dihydricphenol/min
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Control
Mycorrhiza
60 days
90 days

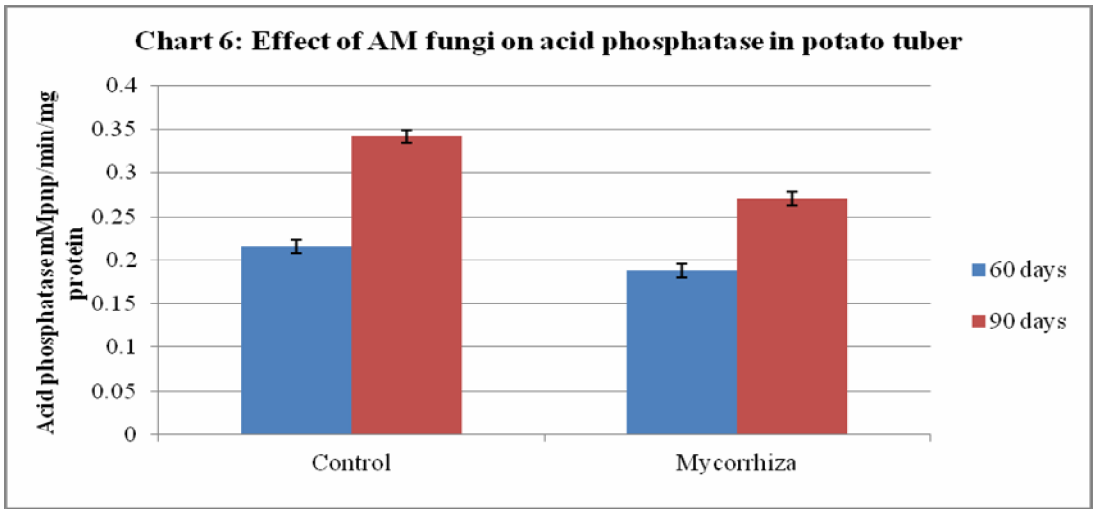
Chart 6: Effect of AM fungi on acid phosphatase in potato tuber
Acid phosphatase mMpnp/min/mg protein
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
Mycorrhiza
60 days
90 days

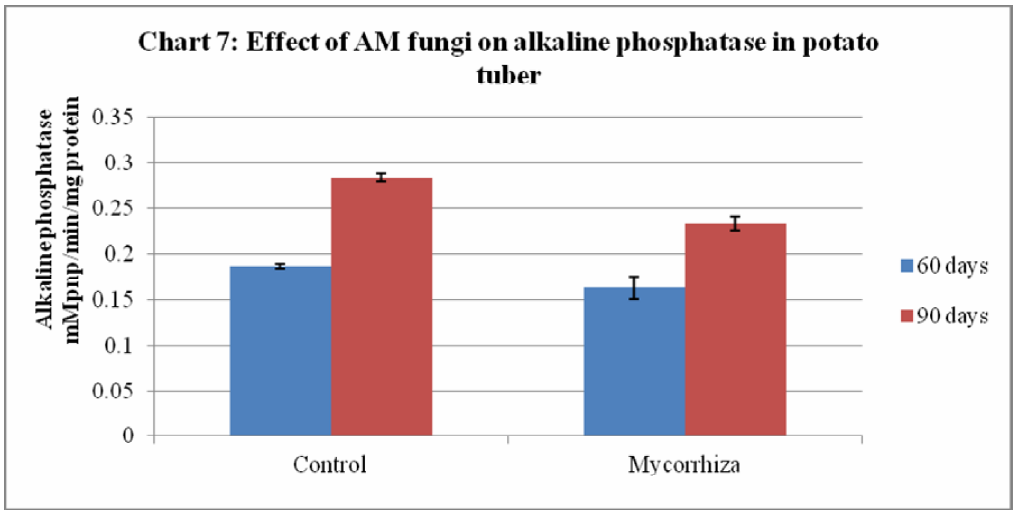
Chart 7: Effect of AM fungi on alkaline phosphatase in potato tuber
Alkaline phosphatase mMpnp/min/mg protein
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
Mycorrhiza
60 days
90 days

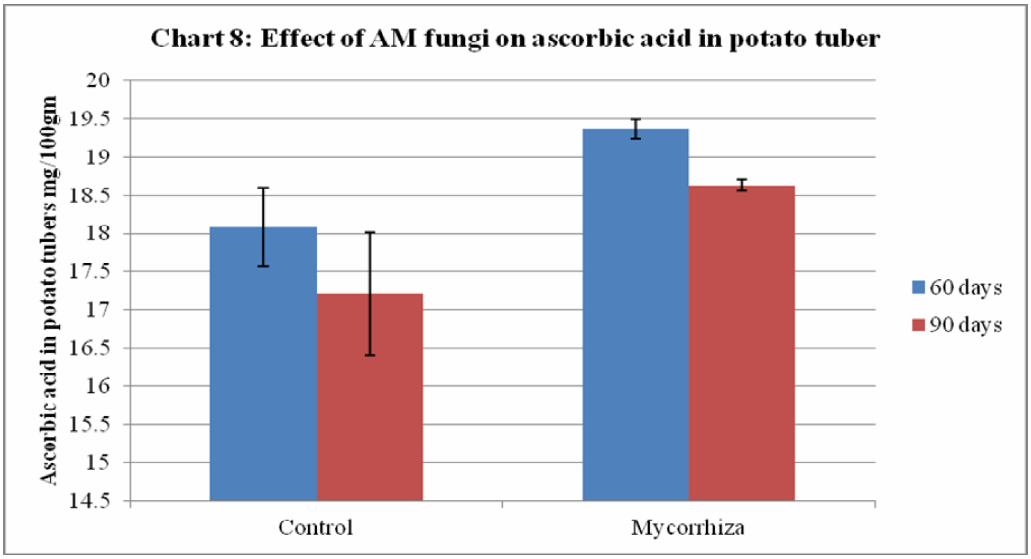
Chart 8: Effect of AM fungi on ascorbic acid in potato tuber
Ascorbic acid in potato tubers mg/100gm
20
19.5
19
18.5
18
17.5
17
16.5
16
15.5
15
14.5
Control
Mycorrhiza
60 days
90 days

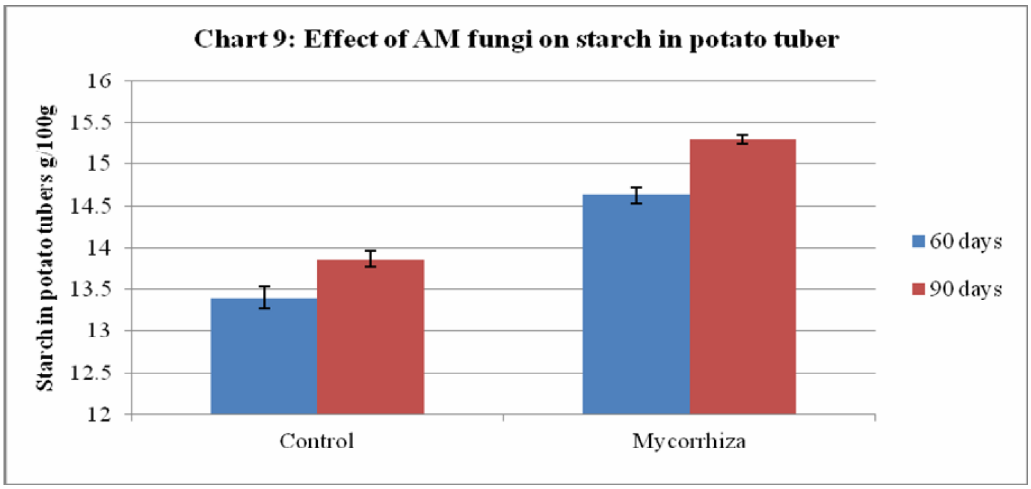
Chart 9: Effect of AM fungi on starch in potato tuber
Starch in potato tubers g/100g
16
15.5
15
14.5
14
13.5
13
12.5
12
Control
Mycorrhiza
60 days
90 days

Allen *et al.,* (1981), Kucey and Paul, (1982) Snellgrove *et al.,* (1986) and Bhosale and Shinde (2011) have suggested that the rate of photosynthesis is higher in mycorrhizal plants compared to non-mycorrhizal plants. Kim *et al.* (2010) during their study on *Capsicum annuum* reported a remarkable increase in chlorophyll content in plants inoculated with AM fungi and *Methylobacterium oryzae*. Our results suggest a comparatively higher amount of chlorophyll a, chlorophyll b and total chlorophylls being in agreement with these studies.

Protein content of potato tubers also depicted a considerable difference in plants grown in the presence of AM fungi and those grown in its absence. Mycorrhizal plants contained higher protein levels than non-mycorrhizal plants. Our findings of increased protein content were similar to the results obtained by Lenin *et al.* (2010) when they studied four different vegetable crops (Chart 2). Plants when analyzed for estimation of total carbohydrates. The content of total carbohydrates was greater in mycorrhizal plants than non-mycorrhizal plants. Wu and Xia (2006) studied the effect of AM fungi on various parameters in *Citrus* and obtained results that indicated higher levels of total carbohydrates in mycorrhizal plants than the plants grown in their absence (Chart 3).

Amount of polyphenol oxidase and peroxidase was estimated and results showed higher levels of both enzymes in mycorrhizal plants as compared to control plants indicating mycorrhization in colonized plants. Our results on the higher levels of peroxidase enzyme was concomitant with the results obtained by Latef and Chaoxing (2011) when they performed salinity related experiments on Tomato plants. Higher levels of polyphenol oxidase and peroxidase in mycorrhizal plants was also observed by Nisha and Sevananrajeshkumar (2010) when they performed experiments with 7 types of AM fungi and their effect on *Wedilla* (Chart 4 and 5). Alkaline and acid phosphatase enzyme levels displayed a slightly different trend as compared the other biochemical parameters (Chart 6 and 7). Mycorrhizal plants contained low levels of these enzymes, while control plants possessed greater quantities of the same. These enzymes are involved in phosphate synthesis and so a scarcity of phosphorus stimulates buildup of these enzymes; in abundance of the element, levels of phosphate synthesizing enzymes falls drastically. The amount of alkaline phosphatase in control plants that were 90 days old contained 0.284±0.0040 mM pnp/min/mg protein and the amount of acid phosphatase was 0.342±0.0075 mM pnp/min/mg protein. In case of mycorrhizal plants the amount of alkaline phosphatase was 0.233±0.0076 mM pnp/min/mg protein and the amount of acid phosphatase 0.271±0.0076 mM pnp/min/mg protein. Our results shared similarity with the work carried out by Nisha and Sevananrajeshkumar (2010).

After estimation of the ascorbic acid content, it was observed that 18.083±0.5204 of ascorbic acid was present in tubers of control plants and 19.366±0.1258 was present in tubers of mycorrhizal plants after 60 days. This level increased to 17.216±0.7973 after 90 days in control plants and to 18.633±0.0763 in mycorrhizal plants (Chart 8). Starch is one of the major biochemically important components in a potato tuber. It was observed that mycorrhizal plants contained higher levels

of starch than non-mycorrhizal plants. Wu and Xia (2006) also obtained similar results when the amount of starch was observed to greater in mycorrhizal plants (Chart 9).

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