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EFFECT OF BIOSTIMULANTS ON GROWTH, YIELD AND QUALITY OF BELL PEPPER CV. YOLO WONDER

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Bell pepper (*Capsicum annuum*) has been in high demand since ever. High quality of fruit and increased yield are desired characters both for the researchers and the growers. The impact of foliar application of chitosan (CHT), Salicylic Acid (SA) and Putrescine (PUT) on fruit yield and physico-chemical characteristics of "Yolo Wonder" bell pepper cultivar was studied for two consecutive years (2012 and 2013). Results indicate application of chitosan (0.5), putrescine (3 mM) and SA (3 mM) increased fruit weight, fruit diameter, and yield as compared to control and other treatments. Fruit firmness was increased in PUT (2 mM, 3 mM) and SA (3 mM) as compared to control. Furthermore, fruit harvested from these treated plants did not affect days to fruit maturity and number of fruits per plant. Higher PUT (3 mM) and SA (3 mM) concentration proved significantly better in reducing disease incidence. Present research proves SA and PUT sprays more effective than CHT for improving fruit physical and chemical characteristics such as fruit size, fruit weight, yield, fruit firmness, and vitamin C content. Finally, the biostimulants used in this study can easily be used to improve yield and quality of bell pepper without compromising the food safety standards.

Keywords: *Capsicum annuum*, GRAS chemicals, Productivity, physico-chemical characteristics, growth elicitor

INTRODUCTION

Bell pepper (*Capsicum annuum*) is the most important crop in solanaceous family after potato and tomato (Mahmood *et al*., 1999) and is famous throughout the world due to its high nutritional value (Kelley and Boyhan, 2009). In Pakistan, bell pepper is popular crop among farmers of peri-urban areas around big cities. While area and production under bell pepper is 66500 ha and 191800 tons respectively (GOP, 2010). Bell pepper is such an important food crop that its improved quality and production is of prime interest. Yield and quality of fruit depends on various pre- and postharvest factors which include environmental conditions, harvest maturity and post-harvest factors (Serrano *et al.*, 1996). Many research groups are struggling to enhance quality and yield of bell peppers. For this purpose chemicals and hormones had been in use for many decades but now round the globe health concerns are increasing among people. Demand for chemical free and safe food is increasing day by day. These emerging concerns are leading towards use of biostimulants which are food grade chemicals and also known as GRAS (Generally regarded as safe) chemicals for producing food crops. Although significant amount of work has been reported on enhancement of quality of bell pepper through use of mineral nutrition, there are very few reports on the use of biostimulants to enhance the yield and quality of the bell pepper fruit. GRAS chemicals are not only capable to enhance yield and quality of fruits and vegetables but they also improve plant health by controlling diseases.

Chitosan (CHT) is a biodegradable compound found naturally and is derived from crustaceous shells. Chitosan has proven to be beneficial to control several diseases of horticultural crops both in pre and postharvest stages (Banos *et al*., 2006). Foliar-applied chitosan increased plant growth, yield and quality of bean plants (Sharifa, 2013), radish (Farouk *et al*., 2008) and cucumber (Ghoname *et al*., 2010). Salicylic acid (SA) is one of the most important phenolic compounds, found in several plants. It is considered a hormonal substance which plays a vital role in regulating plant growth and development (Wang *et al*., 2006). SA is also effective to regulate important physiological processes of plants like growth and development, membrane permeability, ion uptake and transport (Simaei *et al*., 2012). It acts as bio-messenger or signaling agent in plants which promotes tolerance against several biotic and abiotic stresses (Horvath *et al*., 2007). Low concentration of salicylic acid has indicated increase in yield and quality of strawberry plants (Kazemi, 2013). Salicylic acid can also play a significant role in plant water relations, photosynthesis and growth (Arfan *et al*., 2007). Plants of cucumber and tomato when sprayed with lower concentrations of SA showed

significant increase in fruit yield (Larque-Saavedra and Martin-Mex, 2007).

Polyamines (PAs) are positively charged small aliphatic amines and are included in GRAS (generally regarded as safe) chemicals. They are found abundantly in living organisms. Foliar application of putrescine (as one of the polyamine group) resulted in significant increase of vegetative growth (including plant height, number of leaves, number of branches and fresh weight of plants) and yield of eggplant compared to control plants (El-Tohamy *et al.*, 2008). Role of putrescine for improving plant growth and development by increasing cell division and differentiation was obvious in bean plants (Altman *et al*., 1982). Putrescine has a significant role in stimulating growth and enhancing productivity of many plants like sweet pepper (Talaat, 2003), tomato (Cohen *et al*., 1982) and pea plants (Gharib and Hanafy, 2005).

Keeping in view the effectiveness of chitosan, salicylic acid and putrescine treatments in fruits and vegetables, this study was conducted to evaluate the efficiency of these bio-stimulants to enhance the yield and quality of *Capsicum annuum* "Yolo Wonder" cultivar.

MATERIALS AND METHODS

The present research was conducted at Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. The latitude and longitude of experimental area is 33° 40' N, 73° 10' E. Monsoon rains at Rawalpindi come in July and August with monthly average of 200 and 225 millimeters respectively. The coldest month is January when mean minimum temperature is 3°C. June is the hottest month with the mean maximum temperature of 40°C. Different concentrations of chitosan (0.3%, 0.4% and 0.5%), putrescine (1mM, 2mM and 3 mM) and salicylic acid (1 mM, 2 mM and 3 mM) as foliar sprays were applied to bell pepper cv. "Yolo Wonder". The concentrations of each chemical were sprayed separately to the plant leaves and fruits with a commercial hand held sprayer. Sprays were repeated at weekly intervals starting from two weeks after transplanting. Calcium Chloride 0.6% was applied to all treatments including control. The number of sprays per plant was kept constant and a total of seven sprays were applied, ensuring that each plant receives complete coverage on abaxial and adaxial surfaces of leaves and fruits. Each treatment had 60 plants and was replicated thrice. Effects of all the treatments were compared with control which received no treatment but calcium chloride 0.6%.

Following parameters were studied:

***Plant physical characteristic*:** Leaf area of the plant was recorded with the help of leaf area meter (Model AM 100-002). Plant height and number of diseased fruits per plant was recorded at the day of harvest with the help of measuring tape. Days to bloom and maturity was calculated from the day of transplanting in the field.

***Fruit physical quality characteristics*:** Sample of 10 mature fruits was taken for measuring the physical quality parameters like diameter (cm), weight (g.), marketable yield in tons per ha. While fruit firmness was determined hedonically using score from1 to 9 (1 = extremely soft to 9 = extremely hard).

***Fruit chemical quality characteristics*:** The same fruits used to determine the fruit firmness were sliced and fruit juice was extracted for analysis of total sugars, total soluble solids and titratable acidity. Total soluble solids (TSS) were measured according to AOAC (1990) using hand refractometer at room temperature. Total sugars were determined using method as described by Hortwitz (1960). Ascorbic acid was measured according to the method described by Hans (1992).

RESULTS

***Plant physical characteristics*:** Significant increase in plant height and leaf area was noted in fruit treated with chitosan, putrescine and salicylic acid sprays (Table 1). The increase in the plant height and leaf area was proportional to the concentration of the chemicals applied. Maximum plant height (44. 17 cm and 43. 55 cm) was recorded in SA 3 mM treatment followed by putrescine (3 mM and 2 mM) treatment for both the years under study, while lowest value of height (36cm and 35.11cm, respectively) was recorded in untreated plants. Other treatments showed the intermediate results (Table 1). The result for leaf area also has more or less similar trend with maximum value in SA 3 mM treatment followed by putrescine 3 mM treatment during 1st year while putrescine 2mM during 2nd year of the study (Table 1). Different treatments applied to bell pepper "Yolo Wonder" did not affect days to bloom significantly, as evident from the data presented in the Table 1. Data also shows that treatments of chitosan, putrescine and salicylic acid did not affect the days to fruit maturity.

***Fruit physical characteristics at harvest*:** Fruit weight (total and average), and fruit diameter were significantly increased in plants treated with different chemicals (Table 2). A significant increase in fruit weight was recorded during both the years by all treatments compared to control plants. Highest total fruit weight was recorded in the plants sprayed with 3 mM salicylic acid followed by the plants received 3 mM putrescine treatment. In case of average fruit weight, SA 3 mM and putrescine 3 mM showed highest values in both years and were statistically at par. A substantial increase was observed in fruit diameter during both the years of study by all treatments of bio stimulants as compared to control (untreated). Maximum fruit diameter was (56.57 and 54.83) recorded in SA 3 mM treatment followed by putrescine 3 mM and 2 mM respectively for both the years

Table 1. Vegetative and reproductive growth of "Yolo Wonder" bell pepper affected by different treatments of biostimulants.

Treatments	Plant Height (cm)		Leaf Area (cm2)		Days to Bloom		Days to Fruit Maturity	
	2012	2013	2012	2013	2012	2013	2012	2013
Control	36.000D	35.11C	24.68EF	24.637E	40.00A	39.33A	68.33A	69.33A
CHT 0.3%	39.47C	39.74B	23.41F	23.66E	40.33A	39.67A	68.67A	70.00A
CHT 0.4%	39.89C	40.78AB	23.93EF	23.29E	40.33A	39.67A	69.00A	69.33A
CHT 0.5%	41.11BC	39.67B	25.95DE	27.34D	39.67A	39.00A	68.67A	69.67A
PUT 1 mM	41.28BC	40.88AB	24.92EF	24.17E	41.33A	39.33A	69.67A	69.33A
PUT 2 mM	42.83AB	42.44AB	30.26B	32.44AB	40.33A	39.00A	69.33A	69.67A
PUT 3 mM	41.00BC	43.00AB	33.78A	30.33BC	39.33A	39.67A	68.67A	69.67A
SA 1 mM	40.38C	40.44AB	27.61CD	27.72D	39.33A	38.67A	68.67A	68.33A
SA 2 mM	41.34BC	41.44AB	29.61BC	29.44CD	40.33A	39.33A	70.67A	69.67A
SA 3 mM	44.17A	43.55A	33.95A	34.61A	40.00A	39.00A	69.00A	70.33A
LSD	2.41	3.42	2.41	2.59	2.73	1.84	2.43	2.45

Means within a column having same letters are statistically non- significant using Least Significant Difference Test.
Means separated within columns using LSD test (5%level).

Table 2. Effect of different concentrations of biostimulants on physical characteristics of "Yolo Wonder" bell pepper.

Treatments	Total Fruit Weight (g)		Average Fruit Weight (g)		Average Fruit Dia (mm)		No. of Fruit Per Plant	
	2012	2013	2012	2013	2012	2013	2012	2013
Control	339.02E	413.68F	21.64F	28.24F	46.79B	46.48E	15.67A	14.67A
CHT 0.3%	457.06B	439.38DEF	31.23CD	29.98DEF	54.16AB	48.20DE	14.67A	14.67A
CHT 0.4%	409.80CD	432.44EF	27.38DE	28.22F	49.89AB	47.22DE	15.00A	15.33A
CHT 0.5%	376.08DE	449.15CDE	24.05EF	28.60EF	55.56AB	47.92DE	15.67A	15.67A
PUT 1 mM	439.22BC	480.77C	28.19DE	32.91CD	53.94AB	51.20BC	15.67A	14.67A
PUT 2 mM	539.41A	520.51B	34.60BC	34.07BC	55.78AB	53.58AB	15.67A	15.33A
PUT 3 mM	560.43A	549.15AB	35.83AB	37.49A	56.42A	53.830B	15.67A	14.67A
SA 1 mM	411.76CD	429.49EF	28.11DE	29.33EF	49.15AB	48.11DE	14.67A	14.67A
SA 2 mM	459.80B	466.67CD	30.75CD	31.86CDE	56.33A	49.53CD	15.00A	14.67A
SA 3 mM	560.98A	569.66A	39.32A	37.18AB	56.57A	54.83A	14.33A	15.33A
LSD	39.56	32.89	4.18	3.29	9.034	2.83	1.96	1.25

Means within a column having same letters are statistically non- significant using Least Significant Difference Test.
Means separated within columns using LSD test (5%level).

under study (Table 2). The comparison of the treatments regarding fruit diameter showed that all the treatments were statistically non-significant with each other during first year of study (2012), while during second year (2013), there was a clear difference amongst the treatments where SA (3 mM) and Put (2 mM and 3 mM) proved statistically superior than other treatments. The research data remained non-significant as for as number of fruits per plant is concerned during both the years by sprays of different bio-stimulants.

Fruit firmness was also significantly high during both seasons compared to control (Table 3). Maximum firmness was observed in higher concentrations of PUT (2 mM and 3 mM) and SA (3 mM). While lowest score for fruit firmness was recorded in control (7.76 and 7) during both the years under study.

The data regarding the diseased fruits per plant given in Table 3 reveals that plants treated with different bio stimulants got significantly low disease incidence on fruits as compared to the untreated plants (control). During first year SA 3mM proved the best treatment followed by SA 2mM. While during the second year PUT 3mM produced the best results followed by SA 3mM and SA 2mM which remained equally effective.

***Marketable yield*:** The effect of all the treatments on yield per hectare of bell pepper cultivar 'Yolo Wonder' given in Table 3 shows that most of the treatments were effective in increasing fruit yield compared to untreated plants (control). Maximum fruit yield was achieved in case of salicylic acid 3 mM, followed by Putrescine 3 mM and 2 mM treatments. On the other hand lowest yield was recorded in the plants which were kept in control plots and received no treatment.

***Chemical analysis of fruit*:** Figures shown in Table 4 reveal that total sugars and total soluble solids (TSS) contents were increased significantly by different treatments of bio-

Table 3. Effect of different biostimulants on firmness, yield per ha and disease incidence in "Yolo Wonder" bell pepper.

Treatments	Fruit Firmness (score)		Yield (tons per ha.)		Disease incidence	
	2012	2013	2012	2013	2012	2013
Control	7.77D	7.00D	9.73E	11.87F	5.33A	5.67A
CHT 0.3%	8.33BC	7.33CD	13.11B	12.61DEF	3.67BCD	4.67B
CHT 0.4%	8.30BC	7.33CD	11.76CD	12.41EF	4.67AB	4.67B
CHT 0.5%	8.50AB	7.50CD	10.79DE	12.89CDE	4.33ABC	4.33BC
PUT 1 mM	8.33BC	7.83BC	12.60BC	13.79C	5.33A	4.67B
PUT 2 mM	8.83A	8.17AB	15.48A	14.93B	4.00BC	4.00BCD
PUT 3 mM	8.83A	8.67A	16.08A	15.76AB	3.33CDE	3.33D
SA 1 mM	8.00CD	7.50CD	11.81CD	12.32EF	4.00BC	4.67B
SA 2 mM	8.33BC	7.67BC	13.19B	13.39CD	2.67DE	3.67CD
SA 3 mM	8.77A	8.50A	16.09A	16.34A	2.33E	3.67CD
LSD	0.41	0.54	1.13	0.94	1.20	0.98

Means within a column having same letters are statistically non- significant using Least Significant Difference Test. Means separated within columns using LSD test (5%level).

Table 4. Effect of different treatments of biostimulants on TSS, total sugar and ascorbic acid contents of "Yolo Wonder" bell pepper

Treatments	TSS (°Brix)		Total Sugars (%)		Ascorbic Acid (mg/100g)	
	2012	2013	2012	2013	2012	2013
Control	5.03F	5.30C	4.10D	3.72C	46.76E	40.72B
CHT 0.3%	5.57E	6.10BC	4.27CD	4.49BC	49.33D	50.03A
CHT 0.4%	5.70DE	5.83BC	4.10D	4.18BC	50.92CD	49.89A
CHT 0.5%	6.43BC	6.40AB	4.37CD	4.34BC	50.48CD	51.60A
PUT 1 mM	6.07CD	6.47AB	4.70BC	5.94A	54.10AB	52.09A
PUT 2 mM	5.83DE	6.03BC	5.80A	6.03A	52.71ABC	51.18A
PUT 3 mM	6.80AB	7.20A	6.03A	6.09A	54.86A	52.33A
SA 1 mM	5.63DE	5.87BC	5.10B	5.76A	51.99BC	48.71A
SA 2 mM	5.97CDE	6.13BC	5.70A	5.16AB	52.24BC	52.24A
SA 3 mM	6.97A	7.23A	5.97A	6.03A	52.28ABC	52.28A
LSD	0.48	0.93	0.44	1.08	2.578	5.66

Means within a column having same letters are statistically non- significant using Least Significant Difference Test. Means separated within columns using LSD test (5%level).

stimulants. The highest amount of total soluble solid values were recorded in fruits treated with 3 mM PUT and 3 mM SA where both treatments remained at par during both the years. The data of total sugars show that it was also significantly increased by sprays of different treatments when compared to untreated plants (Table 4). Total sugar contents of fruits treated with chitosan remained unaffected. Higher values of total sugar contents were recorded in the fruits which received the sprays of 3 mM putrescine followed by 2 mM putrescine and 3 mM salicylic acid treatment. The lowest value for total sugars of bell pepper fruit juice was recorded in control.

Ascorbic acid contents of bell pepper were also increased significantly in treated plants. During the 1st year study, effects of PUT and SA were more promising than all other treatments in enhancing ascorbic acid contents. PUT 3 mM and SA 3 mM proved to be the best treatments in increasing ascorbic acid contents of bell pepper fruit. Lowest values for ascorbic acid were noted in the fruits which were not treated with biostimulants. During the 2nd year of the study all applied treatments remained at par with each other and their effects on ascorbic acid contents were alike (Table 4) but were significantly higher when compared with the control.

DISCUSSION

In the present study plant physical characteristics like plant height and leaf area were significantly affected by the application of biostimulants. The general positive effect of these biostimulants was proportional to their concentrations i.e., more plant height and leaf area was recorded under higher concentrations of these growth eliciting substances. SA and putrescine have been reported to improve the

vegetative parameters of different plants including eggplant and basil plant (El –Tohmy *et al*., 2008, Gharib, 2007).

As polyamines are effective for enhancing cell division and differentiation, hence, they are known as growth stimulators. Such findings were confirmed in bean plants (Altman *et al*., 1982). Abd El-Wahed *et al*., (2005) found that spermidine (polyamine) significantly enhanced plant height, number of branches, shoot, fresh and dry weight per plant, during vegetative and flowering stages in chamomile plants. Moreover, putrescine has a regulatory role in promoting productivity of many plants such as sweet pepper (Talaat, 2003), pea plants (Gharib and Hanafy, 2005) and tomato (Cohen *et al*., 1982). It is an admitted fact that plant growth is supported by the organic carbon source which can alter the growth rate while its direction is regulated by plant growth regulators (Jimenez-Cervantes, *et al*., 1998). The growth promoting effect of polyamines is considered because of their contribution to cellular carbon and nitrogen to the plants. Secondly, they are cationic molecules, positively charged under intracellular pH, which is helpful in plant growth and development. According to some researchers the increment effect of polyamine on growth rate is because they help in the uptake of minerals like N, P and K from soil (Shawky, 2003). The role of polyamines in plant growth and development has been studied by several researchers. Exogenous application of putrescine increased vegetative growth of gladiolus plant (Nahed *et al*., 2009). Comparable effects of polyamines were reported by Shawky (2003) who found the increase in the growth and leaf nutrients uptake in pepper. Putrescine treated trifoliate orange seedlings showed significant increase in shoot and root biomass (Wu et al., 2010). Application of PUT and SPD enhanced the growth of oranges by helping in the uptake of nutrient elements from the roots (Qing-Sheng and Zou, 2009). Putrescine has also been found to be a substitute for inorganic nitrogen for the growth of in vitro explants from dormant tubers of *Helianthus tuberosum* (Evans and Malmberg, 1989).

The stimulatory effect of SA on different characteristics of sweet pepper growth could be attributed to the positive effects of these components upon the photosynthetic pigments, minerals and bio constituents, endogenous phytohormones and activity of antioxident enzymes (El-Yazeid, 2011).

Growth elicitors i.e. chitosan, putrescine and salicylic acid when used as foliar application significantly improved fruit physical characteristics like fruit weight and diameter. Similar results have been reported in previous studies that SA and PUT improved the physical characteristics of the stone fruits (Ali *et al*., 2014). Better physical characters of fruits as result of salicylic acid and putrescine might be due to their ability to enlarge cell size and strengthening of carbohydrate sink, leading to increased fruit size and weight. For these properties, it has been reported that growth eliciting substances are vital for cell growth and differentiation. It has also been reported that their concentration in cells increases with increased cell proliferation rate (Valero *et al.,* 2002).

Table 3, reveals that plants treated bared significantly less number of diseased fruits as compared to the untreated plants (control) during both the years. During the first year SA 3mM proved the best treatment followed by SA 2mM. While in the second year PUT 3mM produced the best results followed by SA 3mM and SA 2mM which remained equally effective. The protective effects of SA are consistent with previous observations in other plants (Zhang *et al.,* 1999). Murphy *et al*. (2000) reported that application of SA to tobacco could provide a significant degree of protection against *Botrytis cinerea.* Poole and McLeod (1994) found that pre-harvest SA application provided protection for kiwifruit against disease.

Salicylic acid (SA) is a signaling molecule naturally found in plants and involved in the plant defense-related actions against infection by various pathogens. Among the diversified actions of SA in plants, its most important role is as an endogenous inducer of plant defense mechanism against the pathogen attacks.

Significant improvement has been observed by application of different food grade chemicals in fruit firmness. Higher concentrations of putrescine and salicylic acid proved better in this regard. The general positive effects observed during this study on the fruit firmness of bell pepper as a result of applying PUT and SA could be attributed to the characteristics of these bio-stimulants to rigidify the cell structure by making cross links with pectic substances as reported in plums and peaches (Khan *et al*., 2007). Putresine and salicylic acid have shown similar effects on fruit firmness at the time of harvest reported in several other fruits (Ali *et al*., 2014). Application of phytochemicals has increased the yield of bell pepper crop possibly due to the role of these chemicals in improving the development of fruits (Galston *et al*., 1997). PAs are also known as polycationic nitrogenous and anti-senescence compounds (Aziz *et al*., 2001). It is very likely that these bio-regulators play a vital role in fruit growth and development especially at cell division stage, consequently affecting size and weight of fruit and ultimately the yield of bell pepper crop.

The treatments have significant effect on soluble solids and total sugar contents of bell pepper fruit at harvest. Increase in total soluble solids in sweet pepper fruits were also observed by Abou El-Yazeid (2011). Salicylic acid reported to improve TSS in various fruits including Chinese water chestnut (Peng and Jiang, 2006), strawberry (Karlidag *et al*., 2009) banana fruits (Srivastava and Dwivedi, 2000) and apple (Han and Li, 1997). Putrescine shows different results for different horticultural crops. Contrary to present results, PUT has reduced total sugars and TSS in apple (Costa *et al*., 1986). While, in litchi acidity was increased, total sugars and

sugar to acid ratio was decreased when low concentration of PUT (0.01 mM) was applied (Mitra and Sanyal, 1990). The present study shows that the putrescine treatments had a significant effect on sugar contents of bell pepper, which again proves that putrescine behaves differently in different crops.

Increased ascorbic acid content in treated fruit may be attributed to the suppression of ascorbate oxidase activity in fruit. Results of present study confirm the findings of Peng and Jiang (2006), who reported that SA application to fresh-cut Chinese water chestnut enhanced the ascorbic acid content compared to control. Similarly, Karlidag *et al*. (2009) reported strawberry and tomato fruits with higher ascorbic acid concentrations when treated with SA. Higher endogenous concentration of PUT in bell pepper fruit has been associated with higher ascorbic acid level (Yahia *et al*., 2001).

***Conclusion*:** In general, the results showed positive influence of all sprayed bio-stimulants on the quality of bell pepper plants and fruit. Chitosan, putrescine and salicylic acid sprays had better effect than untreated plants. Application of Salicylic acid and putrescine improved fruit physical and chemical characteristics such as fruit size, fruit weight, yield per hectare, fruit firmness, and vitamin C content. It can, therefore, be concluded that bio-stimulants can easily be used to improve yield and quality of bell pepper "Yolo Wonder". This may lead to supply of safe and high quality fruit to the market.

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