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Post Harvest Preservation of Pointed gourd Fruits with PGPR to Ensure Food Safety

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Abstract

Pointed gourd (Trichosanthes dioica Roxb.) is a major summer vegetable grown all over India and in most parts of Indian subcontinent.The crop has primarily originated from Assam-Bengal region of India. Using high yielding varieties as root cuttings, adequate proportion of fertilizers, economic exploitation of land have appreciably improved growth and yield of the crop. Lack of suitable methods for enhancement of shelf life leads to huge economic losses due to withering, rottening, and thus loss of marketable status. Efforts have been made since 1990 to retain the greenness and achieve non-shrinkage of fruits using growth regulators and chemicals like sodium benzoate (200ppm) ,potassium metabisulphite,(1900 ppm),citric acid(100ppm) .The application of such organic chemicals as well as the growth regulators for prolonging freshness of fruits posed to be uneconomical and ineffective in agricultural system of the developing nations like India due to ignorance and lack of proper training of the peasants. Besides the toxicity of some chemicals viz sodium hypochlorite and chlorine respectively In our present study we have aimed at maintaining marketable status of fruits using Plant Growth Promoting Rhizobacteria (PGPR) in combination with kinetin and gibberellic acid to ensure biosafe food from the health standpoint that has yielded significant results.

Keywords: Enhancement , Growth regulators, Pointed gourd, Plant growthpromoting rhizobacteria (PGPR), Shelf –life.

Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.), a member of Cucurbitaceae (Chakraborty,1982)[5] is a chief vegetable grown during the summer and rainy season all over India and Bangladesh(Pandit et al .,1994)[17].In India ,it is largely grown in Uttar Pradesh , Bihar, Madhya Pradesh, Maharashtra in Central India; West Bengal , Orissa, Assam, in eastern India ;Gujarat in the west and in some parts of Andhra Pradesh , Tamil Nadu in the south.(Bose et al., 1986)[3].De Candolle(1882)[8] reported in his "Origin of Cultivated plants'' that the species of *Trichosanthes* have originated from the old world probably from India. Assam-Bengal region is believed to be the primary centre of origin of pointed gourd(Choudhury,1979)[7] .

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The tender juicy fruits having a moderate taste and low cucurbitacin content renders it important as a vegetable .The tender shoots are also relished as pot herb by many people . High protein, vitamin A and calcium content have added to its importance (Bose,1986, Hazra, 1999)[12]. Besides the new findings that the leaves of *Trichosanthes dioica* reduce blood sugar levels(Chandrasekar et al. , 1988)[6] have enhanced its position in the vegetable market. This crop is warm loving ; a single crop yields fruit for fairly a long time i.e. from February to September. It can be easily cultivated from root cuttings in a variety of light textured soil having good drainage facility, though a well drained sandy-loam to loam soil is ideal for the growth of the crop. It can tolerate high temperatures of 25^0 C-35^0 C, heavy rainfall and requires very little irrigation .All these conditions have made it suitable for cultivation over a wide area. Besides effective control of the common diseases that largely affect the crop is now known .The maturity index of

fruits and vegetables are to be considered to determine the exact time for fruit harvesting In developing countries post harvest losses are largely due to under utilization of resources. Pre-processing to add value and to avoid losses needs to be done to preserve a higher amount of the yield. Suitable alternative processing methods socio economically tenable and technically acceptable has to be adopted in rural areas for extending longivity of fruits and vegetables . Lack of suitable cost effective and environment friendly means for storage of the fruits after harvest makes the situation grave as it causes huge economic loss and wastage of human labour. .Scalding or blanching in hot water, cooling in trays, sulphiting, sun drying, osmoticdehydration,fermentation are some of the conventional methods for post harvest fruit handling but such methods cannot be applied to soft and tender fruits. Washing of fruits in flowing or carbonated water is believed to reduce microbial count on fruit surface and to retard oxidation damage .For this purpose the microbiological and the sensory quality of washing water should be good and temperature should be lower than 5^{o}C.The recommended quantity of water to be used is 5kg/L (Alzamora et al., 2000) [1b].In many parts of our country water is so scarce that such techniques are almost impracticable. Though fruit dipping in few of the organic chemicals, viz. sodium benzoate(1900ppm) and citric acid(100ppm) for 10 minutes(Chakraborty,1991)[4] and growth regulators like kinetin(50ppm) ,gibberellic acid (GA_3)-20ppm has been recommended by previous workers. (Som et al. 1998)[19] for extending shelf life which should be subsequently followed by air drying and storage in zero energy cool chamber at 27^0 -30^0 C with 94% relative humidity , but such methods do not seem to be commercially viable for large scale use .Besides such treatments enhances shelf life for a short duration of only four days. Thus, keeping these drawbacks in mind we have focused on developing a standard fruit treatment method for required period for extending shelf life .

The objective of this study was to determine whether Plant Growth Promoting Rhizobacteria (PGPR) isolates1, 2and3 and in combination with kinetin (50ppm),ga_3 are capable of increasing shelf life considerably and to make a comparative study between the two treatments with different time and concentration variations for recommending suitable concentrations of PGPR and growth regulators viz. kinetin and GA for commercial application.

Materials and Methods

1. Microbial cultures: Three wild type PGPR strains viz. PGPR1, PGPR2 and PGPR3 isolated from the endophytic regions of pointed gourd crop, grown in two agricultural fields of Nadia district in West Bengal, India .The strains were capable of producing Indole -3-Acetic Acid (iaa) and HCN were used in our experiment. The bacterial isolates were initially isolated in NA media by serial dilution of $1x10^6$. For long term storage the strains were maintained at -80^0C in Tryptic soy broth (TSB) (difco Laboratories, Detroit) with20% glycerol. For experimental use , 24 hours old cultures in 250ml conical flasks containing Luria Bertani (Miller) broth(LB)(SRL,Maharashtra ,India) amended with Tryptophan broth incubated in rotary shaker (150rpm)(Stuart SCIENTIFIC, orbital incubator,SI 50) For 24 hours at 27^0C , were centrifuged at 8000 x g for 10 minutes .Bacterial suspension was discarded and the supernatant was then used for the treatment. The bacterial suspension thus centrifuged was found to have a concentration of $40x10^7$ CFU/ml.

Morphological characterization of the bacterial strains: The isolates obtained from the rhizospheric soil were initially morphologically characterized by studying their colony morphology, gram nature by Gram staining and flagellar motility along with endospore formation by West's method and by staining with malachite green respectively. The isolates were grown in Bacillus differentiation media as well.

Testing isolates for PGPR activities: The bacterial isolates were subjected to PGPR confirmation tests, viz. IAA production and HCN producing abilities of the isolates.

IAA production.

Production of IAA by test bacterial strains were measured following the method of Gordon and Weber (1951).Loopful of isolates were inoculated in 25ml of Luria Bertani broth (Himedia Laboratories,Mumbai,India) amended with 20 ml of 0.2% L tryptophan(Merck,India)The cultures were then incubated for 24 hours at 28^0C on rotary shakers (Orbital ,Stuart Scientific) and centrifuged at 8000xg at 9^0C for about 15 minutes .2ml of the supernatants of each of the isolates were taken and 2-3 drops of o-phosphoric acid were added to each. To each of the tubes 4 ml of Salkowski's reagent (Ehmann, 1977) were added. The samples were incubatedindarkatroomtemperaturefor25minutes.Theabsorbance was read at 530 nm wavelength in spectrophotometer (Cecil).Concentration of auxin produced by the samples were then determined from standard curve using IAA as standard (10-100µg/ml)

HCN production.

The rhizospheric soil bacterial isolates were streaked on King's B medium [13] plates amended with 4.9g/l glycine in such a way that single isolate were placed on each of the plates. Then whatman no.1 filter paper discs (9cm diameter)were soaked in 0.5% picric acid and 0.2%$NaCO_3$and placed in the lid of each petridish resulted in the colour change of the filter paper from deep yellow to orange to orange brown and finally to dark brown (depending upon the intensity of production.).In case of negative response, the deep yellow colour of the filter paper remained unchanged.

2. Plant Material: Young, tender freshly plucked fruits of pointed gourd (Trichosanthes dioica) of the local varietyHaripatkhali and Kajli were first washed with tap

water and air dried followed by washing with sterile distilled water maintaining aseptic conditions. The fruits were then blotted dry with sterile filter papers followed by air drying.

3. Treatment of pointed gourd fruits with organic chemicals viz. citric acid, growth regulators i.e. kinetin and gibberellic acid and PGPR supernatant..

Properly cleaned and air dried fruits were initially weighed and the weights of each set were recorded .Each set for different treatments consisted of four pointed gourd fruits .The experiment consisted of 11 treatments and four replications. Treatments included citric acid (100ppm) ; kinetin (50ppm),GA_3 (20ppm)solutions; $PGPR_1$ ($40x10^7$ CFU), PGPR2($40X10^7$CFU),PGPR3 ($40X10^7$ CFU) ; PGPR1 +Kinetin (50ppm), PGPR2 +Kinetin (50ppm),PGPR3+Kinetin(50ppm) ; non treated control and medium control. The fruits were dipped in the different treatmentsfor30minutes and 60 minutes (citric acid) respectively, (time was optimized after different time variation treatments of different sets with the help of sterile forceps. After such treatments fruits were air dried. Two separate sets were maintained for each treatment in sterile gunny bags measuring (8''x4") and sterile baskets of 5'' diameter respectively. After placing the fruits in the gunny bags the mouths were tied with a thread and the bags labeled accordingly. Such sets were then placed in cold storage conditions of 8^0-10^0C and relative humidity of 85%.(Koley et al. 2009) [14].The fresh weight of each set was recorded after every 24 hours and this was continued for seven consecutive days. Morphological changes of the specimen were noted daily .Mean decrease in weight for each set over the period of seven days were calculated separately for gunny bags as well as basket sets .

4. Estimation of chlorophyll content in fruit skin. 250mg peelings of fruits of pointed gourd from each of the treatments from the gunny bags and the basket sets were taken in clean sterile mortars. The tissues were grinded with a pestle and 5 ml of 80% acetone were added .The solutions were then centrifuged at 5000 rpm for 5minutes and the supernatants transferred to 10ml volumetric flasks. The process was repeated till the pellet became colourless .The mortar and pestles were washed thoroughly with 80% acetone and the washings were added to the volumetric flask , the volumes were adjusted to 10ml.Absorbance of the solutions were read at 645 and663 nm respectively against 80% acetone blank.

The amount of chlorophyll present in the extract was calculated in terms of mg chlorophyll per g of tissue using the following equation [2].

mg chlorophyll a/g of tissue =$12.7(A_{663})$-$2.69\ (A_{645})$ x V/1000xW

mg chlorophyll b / g of tissue= $22.9(A_{645})$-$4.68\ (A_{663})$ xV/1000xW

mg total chlorophyll /g of tissue =$20.2(A_{645})$ +$8.02(A_{663})$ xV/1000XW

Where A=absorbance at specific wavelength

V=final volume of chlorophyll extract in 80% acetone

And W=fresh weight of tissue extracted

Results

Morphological characteristics of the bacterial isolates used.

The bacterial isolates showed the following characteristics-PGPR1 exhibited a small size, creamy-white colour opaque ,circular shape, entire margin, raised colony,PGPR2 revealed a large and spreading, translucent to opaque white ,irregular, raised colony while PGPR3 had a moderate size ,cream colour with reddish tinge ,opaque centrally and translucent at the periphery, irregular outline filamentous margin, convex colony morphology. The diameter of the colonies ranged from 1mm-2.5cm.The isolates were fast growers, sufficient growth was obtained within 24 hours. All of them exhibited a gram positive nature with rod shaped cells respectively; the former showed endospore formation while all of them possessed flagella .The ability of the isolates to grow in Bacillus differentiation medium suggests that they may belong to this genus.

PGPR related activities of the bacterial isolates

Isolate 1 produced about 16µg/ml IAA and was a weak producer of HCN ,while isolate 2 produced 23 g/ml IAA and was capable of producing large amount of HCN (filter paper turned dark brown) whereas PGPR 3 released 30 g/ml IAA and moderate amount of HCN.

Retardation in physiological loss of weight due to various treatments.

Analysis of the data obtained from the experiments performed to evaluate the effectiveness of the different treatments in controlling weight loss revealed that loss in physiological weight on the average was lower in case of fruits maintained in gunny bags as evident from table 1 and 2 respectively .In both the cases, as revealed from the tables, on the average, gibberellic acid (GA) 20 ppm treatment for 30 minutes proved to be most effective in significantly controlling weight loss of fruits ,though citric acid (100ppm) treatment for 60 minutes yielded equally convincing results and the mean diminution in fresh weight was recorded to be 2.19g and 2.33 g for gunny bag and bamboo baskets respectively . Fruits dipped in different PGPR suspensions alone showed relatively poor performance with respect to fresh weight maintenance and their mean weight loss ranged from 4.35-5.25 g in case of gunny bags and 4.96g-5.80 g in bamboo basket sets , though the value was higher than the different controls .Of these three PGPR strains, PGPR 3 was most efficient in lessening physiological weight loss in both the containers followed by PGPR2 and PGPR 1 .At the end of the experiment such treated fruits showed a shriveled

appearance ,loss in greenness to some extent .A combination of PGPR and kinetin was partially effective in reducing weight loss which amounted to 3.38-4.41g mean decrease in weight in gunny bags and 4.6g to 5.36 g in bamboo baskets .In contrast to this the combination sets of PGPR and gibberellic acid exhibited total success in maintaining constancy of fresh weight where the decrease was only 1.25g (PGPR3 +GA)to 1.54g PGPR1+GA) sets maintained in gunny bags which is negligible .The green colour ,smoothness of skin ,turgidity of fruits was as healthy as fresh tender fruits. Firm pulp texture and lower seed hardness was also observed .Here an interesting point to note was on the average this combination of PGPR+GA showed better performance thanGA alone .While fruits kept in bamboo baskets, though the combination of PGPR+GA recorded adequate suitability but displayed lower performance in comparison to GA, where the mean decrease in weight in the former case varied from1.96g (PGPR3+GA) to 2.22g (PGPR1+GA) in contrast to only GA treatment recorded a decrease of 1.8g only .Even in all combinations designed, PGPR3 exhibited higher efficacy.

Chlorophyll content of fruit skin peelings

In a separate study the chlorophyll content of the pointed gourd fruits were determined following the same conditions of storage i.e. gunny bags and bamboo baskets used, the results are represented in table 3 and 4 .The chlorophyll a ,b and total chlorophyll content was recorded to be highest in case of GA treated fruits ,the amount being as high as 21.799mg/g of tissue (chl,a),20.106mg/g of tissue(chl b) and 19.0065 mg/g of tissue(total chlorophyll content) in case of gunny bags and 15.43 mg/g of tissue(chl a),13.236mg/g of tissue (chl.b) and 12.765 mg/g of tissue (total chlorophyll content) in bamboo basket kept fruits .Citric acid treated fruits ranked next to GA in chlorophyll content in both the cases followed by kinetin .In contrast to the physiological weight loss results ,a combination of PGPR +GA)showed poor chlorophyll content at the end of the period that were as low as 7.5486 (PGPR1+GA) to15.6454 (PGPR3+GA) in gunny bag and bamboo set from 5.6500-10.47611mg/g chl.a respectively The amount was relatively higher than the combination treatments of PGPR +kinetin as well as PGPRsuspensiontreatment.

Table1: The decrease in weight of the pointed gourd fruits subjected to different treatments and maintained in gunny bags.

Treatments	Treatment time(mins)	Initial weight(g)	Decrease in weight during the period in days(D)[a]										Mean decrease in weight (g)[b]
			D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	
Citric acid(100ppm)	60	79	2.6	2.8	2.5	2.4	1.9	1.7	2.0	2.1	2.1	1.9	2.19
Kinetin(50ppm)	30	82.3	10.2	5.1s	4.5	3.6	3.5	3.7	1.4	3.9	4.0	4.4	4.45
GA3(20ppm)	30	80.0	2.0	1.8	1.7	1.5	1.4	1.2	1.3	1.4	1.5	1.2	1.60
PGPR1	30	91.4	6.9	5.5	5.8	4.7	4.4	4.2	4.5	4.9	5.6	6.0	5.25
PGPR2	30	89.5	5.0	5.0	4.8	3.7	5.3	5.5	5.6	5.0	4.8	4.04	4.88
PGPR3	30	95.5	5.5	3.9	4.9	4.7	3.5	3.6	3.8	4.0	4.35	4.9	4.35
PGPR1+Kinetin	30	93.0	5.8	4.2	3.2	3.0	4.5	4.8	4.7	4.5	4.4	3.93	4.41
PGPR2+Kinetin	30	77.4	5.8	3.0	2.5	2.6	3.3	4.0	3.8	3.9	3.5	2.0	3.53
PGPR3+Kinetin	30	95.4	5.0	3.0	2.8	2.6	3.4	3.5	3.5	3.4	3.4	3.24	3.38
PGPR1+GA	30	86.5	1.9	1.8	1.67	1.4	1.23	1.33	1.6	1.4	1.5	1.1	1.54
PGPR2+GA	30	88.9	1.89	1.45	1.35	2.45	1.56	2.35	1.45	1.55	1.05	0.50	1.32
PGPR3+GA	30	88.0	1.65	1.54	1.05	1.00	1.15	1.10	1.25	1.20	1.30	1.25	1.25
Medium control	30	80.0	6.4	6.5	5.5	4.90	4.5	5.85	5.65	6.0	5.80	5.80	5.83
Nontreated(water control)	30	91.4	6.9	5.5	5.8	4.7	4.4	4.2	4.5	5.4	6.0	8.0	6.00

[a] Decrease in weight of fruits in different treatments recorded for a period of ten days respectively.

[b]Mean decrease in weight calculated for the period of ten days

Table2: The decrease in weight of the pointed gourd fruits subjected to different treatments and maintained in baskets.

Treatments	Treatment time(mins)	Initial weight (g)	Decrease in weight during the period in days (D)										Mean decrease in weight(g)
			D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	
Citric acid(50ppm)	60	88.2	2.8	2.6	2.5	2.4	1.7	2.0	2.2	2.3	2.4	2.3	2.33
Kinetin(50ppm)	30	85.0	6.6	5.2	4.9	4.5	4.2	3.6	3.9	4.1	4.5	5.99	4.83
GA3(20ppm)	30	99.0	2.0	1.8	1.7	1.5	1.4	1.2	1.4	1.6	1.9	2.3	1.80
PGPR1	30	90.0	7.5	4.6	5.5	6.5	4.9	5.2	5.4	5.6	6.4	5.5	5.80
PGPR2	30	80.0	7.7	4.3	5.5	5.5	4.3	4.9	5.0	5.2	5.1	4.8	5.46
PGPR3	30	95.0	5.0	5.3	4.8	4.4	4.3	4.6	4.9	4.96	5.26	4.9	4.96
PGPR1+Kinetin	30	98.0	5.9	4.9	4.8	5.4	5.8	5.6	5.4	5.36	5.08	5.3	5.36
PGPR2+Kinetin	30	99.0	7.8	3.7	4.2	4.6	5.3	5.2	5.0	4.9	5.19	5.11	5.12
PGPR3+Kinetin	30	86.9	8.5	6.7	4.7	4.0	3.8	3.5	3.6	1.6	3.1	3.64	4.60
PGPR1+GA	30	83.0	1.9	2.0	1.85	1.67	1.8	1.55	2.09	2.35	2.50	4.51	2.22
PGPR2+GA	30	81.0	1.7	1.98	1.65	1.8	1.9	1.50	1.85	1.95	2.04	3.73	2.01
PGPR3+GA	30	98.0	1.5	1.75	1.55	1.55	1.67	1.78	2.0	2.5	2.1	3.2	1.96
Medium control	30	80.0	7.0	6.7	5.87	5.45	5.67	5.5	5.34	4.9	6.23	6.21	5.89
Nontreated(water control)	30	80.4	8.0	3.2	5.5	4.5	3.2	5.5	6.0	8.0	7.0	6.98	6.88

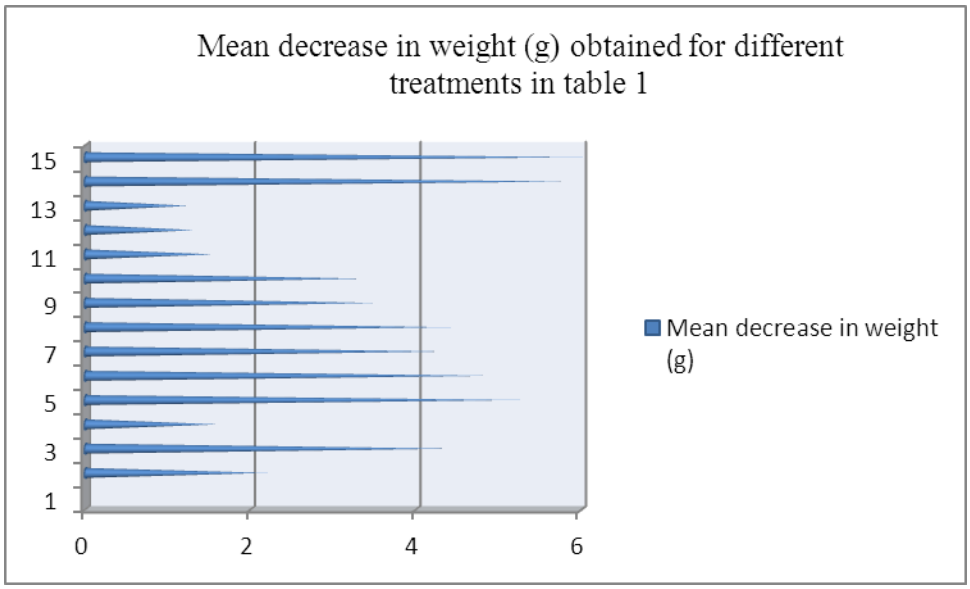
[a] Decrease in weight of fruits in different treatments recorded for a period of ten days respectively.
[b] Mean decrease in weight calculated for the period of ten days

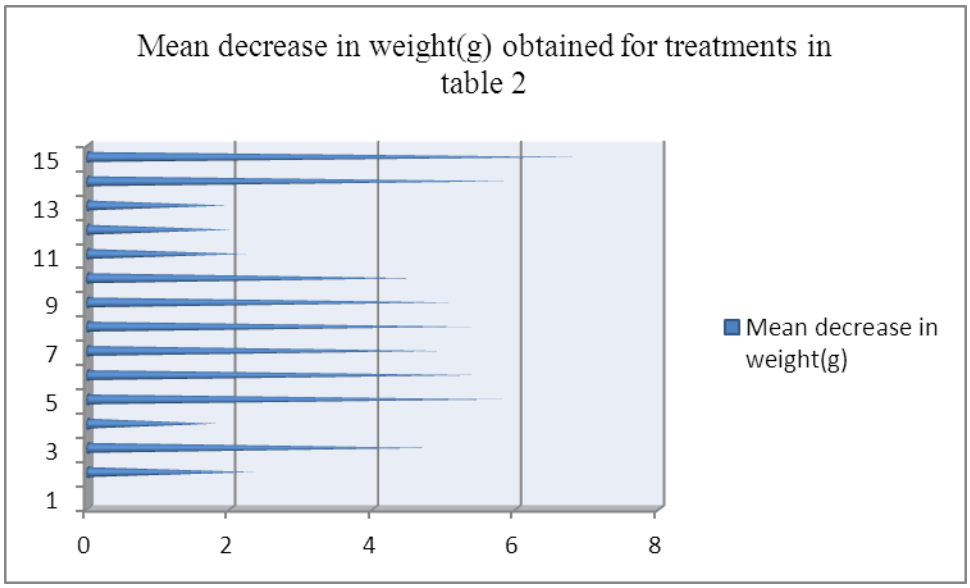
Table3: Chlorophyll content of peelings of different treatments maintained in gunny bag sets

Treatments	Treatment time (mins)	Chlorophylla/unit weight of tissue (mg/g)	Chlorophyllb/unit weight of tissue (mg/g)	Total chlorophyll/ unit weight of tissue (mg/g)
Citric acid(100ppm)	60	20.1103	19.704	18.7650
Kinetin(50ppm)	30	17.999	16.887	15.0985
GA_3(20ppm)	30	21.799	20.106	19.0065
PGPR 1	30	5.4136	3.2806	2.8556
PGPR 2	30	6.7976	3.3748	2.9440
PGPR 3	30	8.9413	6.6904	5.8283
PGPR 1+Kinetin	30	5.8059	4.5372	3.9666
PGPR 2+Kinetin	30	6.8759	3.7218	3.0951
PGPR 3+Kinetin	30	13.3844	9.4272	8.2339
PGPR 1+GA	30	7.5486	5.9998	4.0574
PGPR 2+GA	30	9.9745	7.8465	5.7890
PGPR 3+GA	30	15.6454	13.8494	11.5077
Medium control	30	4.6980	3.6956	2.9987
Nontreated(water control)	30	2.069	1.8604	0.1699

Table4: Chlorophyll content of peelings of different treatments maintained in baskets.

Treatments	Treatment time (mins)	Chlorophylla/unit weight of tissue (mg/g)	Chlorophyllb/unit weight of tissue (mg/g)	Total chlorophyll / unit weight of tissue (mg/g)
Citric acid(100ppm)	60	17.456	16.005	15.000
Kinetin(50ppm)	30	8.9740	6.7122	5.8657
GA_3(20ppm)	30	15.435	13.236	12.765
PGPR 1	30	2.0690	0.2214	0.1699
PGPR 2	30	2.542	2.046	1.7677
PGPR 3	30	3.6905	1.80043	1.54285
PGPR 1+Kinetin	30	4.5679	2.47467	2.1267
PGPR 2+Kinetin	30	6.8340	3.41408	3.03889
PGPR 3+Kinetin	30	6.8789	3.6771	3.1594
PGPR 1+GA	30	5.6500	4.5007	3.7800
PGPR 2+GA	30	7.7500	6.0500	4.955
PGPR 3+GA	30	10.4761	8.8870	6.7654
Medium control	30	1.5560	1.4000	1.5997
Nontreated	30	1.0035	0.9050	0.7990





Discussion

The three PGPR strains used in our experiment were gram positive in nature ,possessed rod shaped cells ,showed flagellar motility ,one of them exhibited endospore respectively.PGPR isolates 1,2 and 3 were selected on the basis of their indole acetic acid producing ability and HCN producing capability .Previously several workers have reported that IAA is produced by different Plant growth promoting Rhizobacteria,viz.*Arthrobacter sp*(Forni,1991)[9],*Pseudomonas putida* (Patten, 2002)[18], *Azotobacter*(Ahmad, 2005). Chakraborty et al 1991[5] have suggested the use of Naphthalene acetic acid (NAA) as fruit dipping treatment for prolonging marketable status of pointed gourd fruits by 3-4 days under ordinary storage conditions.HCN produced by many PGPR ,mainly gram negative bacteria antagonize the growth of several pathogens(Haas et al.2003)[11].The intended purpose of use of HCN in our experiment was to protect the fruits from pathogen attack during storage .Citric acid previously known to protect fruits and preserve them for a considerable period of 7-8 days during storage (Som et al.1998)[19] has also shown a very high response in our experiment too. Neither of the PGPR strains were able to compete efficiently with the growth regulators, kinetin as well asGA_3 in maintaining the healthy status of the fruits significantly .In contrast to this, a combination of PGPR+growth regulators showed quite satisfactory performance. However the best performer was PGPR 3 +GA set in both the storage conditions, though all the PGPR along with GA had registered the lowest decrease in weight .It may be inferred that a cumulative effect of IAA produced by PGPR and GA have triggered such response .This results are in conformity with those obtained by Koley ,2009[14] where application of 100mg/l of NaClO + 500mg/l potassium metabisulphite and 1:10 carnauba wax emulsion diminished physiological loss in weight rate and higher hue angle and chrome values of pointed gourd .Even firm pulp texture and lower seed hardness were retained .Our study further indicated that gunny bag is more suitable matrix for storage of fruits for retaining shelf life than bamboo baskets .It may be due to the fact that it is a confined chamber with adequate arrangement for aeration of tissues to prevent unnecessary loss of moisture by evaporation but allow normal respiration rates. Further study is being carried out for drawing firm conclusion in this regard.

From tables 1 and 2, on one hand and 3 and 4 on the other,it can be concluded that there is a correlation between physiological weight loss of fruits with the chlorophyll content of the same .Merzlyak,1999[16] reported that in senescent tissues degradation of chlorophylls and carotenoids occur simultaneously and only trace amounts of carotene are present in senescent leaves and ripening fruits .The pigment changes during senescence and fruit ripening can be determined from the difference in between reflectance around 680nm and 500nm (R_{680}-R_{500}).This difference increases in such tissues due to decrease in chlorophyll content and increase in car./chl. ratio The fact that considerable amounts of chl. a and b had been obtained quantitively from the fruit peelings indicate that the different treatments with PGPR+growth regulator ,PGPR alone and growth regulators had potentially retarded ageing and senescence of the fruits. It is important to highlight that in all the test conditions chlorophyll a content was relatively higher than b and subsequently both of them higher than total chlorophyll content. Studies with strawberry (a non climacteric fruit like pointed gourd) have unraveled that introduction of synthetic auxins like 1-naphthalene acetic acid and 2, 4-

dichlorophenoxyacetic acid into intact unripe fruits delayed subsequent ripening which could be measured from accumulation of anthocyanins, loss of chlorophyll and decrease in firmness (Given et al 1988)[10] From these findings it may be firmly concluded that the IAA released by our PGPR strains along with the GA added had collectively played a promising role in retarding perishing of fruits in our study.

In a nutshell, the major findings of this study may be summarized as –gunny bags are more suitable for storage and transportation of different cucurbit fruits and vegetables compared to baskets. Though gibberellic acid alone is most effective in retaining freshness of fruits but it is not affordable by a larger section of the farmers, but on the other hand, a dual treatment with PGPR +GA has yielded satisfactory results. Moreover it is a relatively cheaper and environment friendly substitute that does not demand much training and skill for appropriate application. Thus the latter may be recommended for economically tenable sustainable agricultural practices. Critical study is needed to devise a suitable formulation of PGPR +GA. Moreover, the gram positive nature of the isolates, with the rod shaped cellular morphology in all the cases, flagellar motility and presence of endospore followed by ability to grow in Bacillus differentiation medium has a clear indication that the isolates are likely to be species of the genus Bacillus but efforts are being made for accurate identification of the strains.

Future line of research: The present day concepts of water activity of fruits,intermediate moisture food and combined methods of fruit preservation needs to be advocated along with PGPR treatment for fruit preservation for a longer period and retention of healthy ppearanc without loss of palatibility

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