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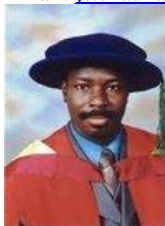


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CHARACTERIZATION OF CRUDE EXTRACELLULAR LIPASE FROM CULTURE BROTH OF *CUNNINGHAMELLA* SP.

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Abstract

Today microbial lipase occupies a position of distinction amongst biocatalysts due to their capability to catalyze a broad range of reactions in water and non water media. In view of the enormous applications of lipases in the industries, lipase produced by *Cunninghamella* sp. in submerged fermentation was partially characterize to determine its suitability in industries. High level of activity of *Cunninghamella* lipase was found at 37 °C and pH 9.0. Stability of lipase was superior within the temperature range of 28 °C to 50 °C and pH 4 to 10. Significant activity at pH 9 to 10 and stability at temperature 50 °C indicates alkaline and thermostable nature of lipase which would determine its use in detergent industries. Among the organic solvents (10% v/v), acetone was found excellent inducer of lipase activity. Monovalent cations (K⁺ and Na⁺) and divalent Ca²⁺ showed stimulatory effect on lipase activity whereas Mn²⁺ comparatively declined lipase activity.

Keywords: Lipase, *Cunninghamella* sp. characterization, thermostable, alkaline.

Introduction:

Lipase (triacylglycerol hydrolase EC 3.1.1.3) are hydrolytic enzymes catalyze cleavage of ester bond of triacylglycerol in watery media and catalyze ester synthesis (esterification) in non watery media (Fernandes et al. 2007; Contesini et al. 2010; Mohamed et al. 2011). Lipases are active at oil-water interface (interfacial activation) and their substrates are water insoluble (Reis et al. 2009). Certain benefits of using lipases for hydrolysis and esterification reactions are: co-factor is not required for their catalytic activity, they carry out catalysis in presence of organic solvents, possess enantioselectivity and they function on broad range of substrates (Hernandez-

Rodriguez et al. 2009). Lipases source may be plants, animals and microbes but microbial lipases have dominated the global enzyme market because of the features they possess (Fernandes et al. 2007; Salihu et al. 2012). Extracellular fungal lipases derived from *Penicillium*, *Aspergillus*, *Rhizopus*, *Fusarium*, *Mucor*, *Cunninghamella verticillata* sp. (Gopinath et al. 2002) have dominated the enzyme market. These genera of fungi are not only factories for the production of lipases but also amylases, cellulases, proteases and xylanases (Savitha and Ratledge, 1991).

Presently lipases have attracted attention for production of biodiesel that is produced by esterification and transesterification reactions on

different types of lipid (Hasan et al. 2006; Gog et al. 2012). Other applications include production of aroma in perfumery industry, as biosensor in diagnostic field, conversion of low quality fat into high quality and cheese ripening in food industry, formulation of detergent, pharmaceutical, oleochemical and dairy industries (Sharma et al. 2016). The major application of lipases in detergent industry in which 32 % of total globally produced lipase is consumed. Detergent industry requires lipases which possess thermostability and catalytic activity in alkaline conditions (Reetz and Jaeger, 1998).

The significance of lipases can be observed by the huge number of published papers in recent times. Actually, over the past certain years, there is gradual enhance in number of publications regarding industrial applications of lipase catalyzed reactions, carried out in various organic solvents. Characterization of lipases is very important which will determine its industrial use. Very few reports are available about production and characterization of *Cunninghamella* lipase. Therefore, the purpose of present study was to partially characterize the crude lipase of *Cunninghamella* sp. to determine its stability at various pH, temperatures, organic solvents and metal ions.

Materials and Methods:

Microorganism and lipase extracellular production

Fungus utilized in this study was previously isolated from soil sample of mustard field and identified as *Cunninghamella* sp. The fungus was

maintained in potato dextrose (PDA) agar slants. For production of extracellular lipase in submerged fermentation, spore suspension from 6 days old slant culture of *Cunninghamella* sp. was prepared and inoculated aseptically in 100 ml of production medium (Xia et al. 2011) of the following composition: 40 g/L bacteriological peptone, 10 ml/L olive oil, 1 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L KH_2PO_4 , 1 g/L $(\text{NH}_4)_2\text{SO}_4$ and 5 g/L sucrose and pH was adjusted to 7.0. Flasks were at 28 °C, 150 rpm for 3 days. At the end of incubation supernatant containing crude protein extract was recovered after filtration and centrifugation of culture broth for removal of mycelium. This crude protein lysate was used for characterization studies of lipase enzyme.

Characterization of lipase enzyme

Determination of temperature optima

Optimum temperature for lipase activity was determined by performing lipase assay at different temperatures (28 °C, 37 °C, 50 °C and 60 °C). Reaction mixture was incubated at different temperature for 30 min followed by estimation of lipase activity by the spectrophotometric method of Winkler and Stuckmann (1979).

Determination of temperature stability

Crude protein extract was pre-incubated at various temperature (28 °C, 37 °C, 50 °C and 60 °C) for 2 h thereafter lipase activity was determined.

Determination of pH optima

For this purpose lipase assay was carried out at various pH (4, 5, 6, 7, 8, 9

and 10). pH of Tris HCl buffer was adjusted in different range and utilized for lipase assay.

Determination of pH stability

Crude protein extract was pre-incubated at various pH (4, 5, 6, 7, 8, 9 and 10) for 2 h thereafter lipase activity was determined. One ml of crude protein extract was mixed with 1 ml of Tris HCl buffer (variable pH) and pre-incubated at optimum temperature for 2 h followed by measurement of lipase activity.

Lipase stability in presence of organic solvents

The following organic solvents were used in the study: methanol, acetone, chloroform and isopropanol. One ml of crude protein lysate was mixed with 1 ml of each of the pure organic solvent and pre-incubated for 2 h at temperature optima thereafter activity of lipase was estimated.

Effect of metal ions on lipase activity

Metal ions utilized at a concentration of 10 mM were KCl, CaCl₂, MnCl₂ and NaCl. Crude protein lysate and metal ions were mixed in equal amount (1 ml of each) and pre-incubated for 2 h at optimum temperature thereafter activity of lipase was estimated.

Results and Discussion:

Effect of temperature on lipase activity and stability

Figure 1 shows that highest activity (150.46 ± 3.73 U/ml) of lipase was obtained at an optimum temperature of 37 °C. Significant lipase activity was also found at 28 °C (121.38 ± 2.65 U/ml) and 50 °C (122.41 ± 4.60

U/ml). Lipase activity was reduced (60.79 ± 4.25 U/ml) when the incubation temperature of lipase assay was 60 °C.

In the temperature stability profile of lipase, enzyme was found stable within the temperature range of 28 °C to 50 °C with maximum activity (189.33 ± 2.32 U/ml) at 37 °C. Thereafter activity slightly declined at 50 °C and reached to minimum (39.30 ± 4.12 U/ml) at 60 °C (Fig. 2). Significant activity and stability of *Cunninghamella* lipase at 50 °C suggest that it can be used in the industries where lipase catalyzed reactions are carried out at higher temperature.

Similar to present results, an optimum temperature of 35 °C was reported by Costa-Silva et al. (2014) for *Cercospora kikuchii* lipase and Sharma et al. (2016) for *A. niger* lipase whereas Oliveira et al. (2014) reported optimum activity of *Candida guilliermondii* lipase at 30 °C. Mase et al. (1995) stated maximum activity of *Fusarium* sp. lipase at 37 °C while optimum activity at 40 °C was reported by Jayaprakash and Ebenezer (2012) for *Aspergillus japonicus* lipase, Zhou et al. (2012) for *Aspergillus oryzae* lipase and Gopinath et al. (2002) for *Cunninghamella verticillata* lipase. Shu et al. (2006) reported that *Antrodia cinnamomea* lipase retained 50 % of its activity within the temperature range of 25 °C to 40 °C.

Colla et al. (2015) reported that *Aspergillus* sp. lipase retained 72% of its activity after incubation at 90 °C for 2 h. Razak et al. (1997) stated that fungal lipases are generally unstable at temperature above 40 °C, opposite to Razak's statement, certain bacterial lipase such as *Bacillus* (Nawani et al. 1998; Ghanem et al. 2000) and

Pseudomonas (Kulkarni and Gadre, 2002) are thermostable over 60 °C. However, lipases secreted by *Aspergillus niger* (Sharma et al. 2016) retained stability above 40 °C, lipase produced by *Rhizopus* sp. (Pastore et al. 2003) retained more than 50 % of its activity when pre-incubated at 50 °C for 1 h and lipase produced from *Geotrichum* sp. (Ginalska et al. 2004) exhibited highest residual activity at 60 °C pre-incubation for 60 min. *Rhizopus* Lipase reported by Iftikhar et al. (2011) retains 80 % of its activity between the temperature 25 °C to 30 °C. Higher optimum temperature 50 °C was reported by Sethi et al. (2016) for *Aspergillus terreus* lipase.

Effect of pH on lipase activity and stability

In the pH activity profile (Fig. 3), maximum activity (135.45 ± 3.02 U/ml) of *Cunninghamella* lipase was obtained when lipase assay was carried out at Tris HCl buffer of pH 9.0. Lipase activity was lowest at pH 4 and 5 (56.04 ± 4.25 U/ml) then it increased with the rise of pH reached to maximum at pH 9.0 thereafter it declined to 112.09 ± 1.37 U/ml at pH 10.0. Lipase activity was higher in alkaline pH range than in acidic pH indicated alkaline nature of *Cunninghamella* lipase.

In the pH stability pattern (Fig. 4), lipase was found stable within the acidic pH range (4-6) with highest stability at pH 5.0 (134.51 ± 3.63 U/ml) thereafter stability declined with rise of pH from 6-7. At pH 9.0 lipase stability again increased to 127.80 ± 4.71 U/ml and then declined towards pH 10.0. In our study lipase activity was higher in alkaline pH range whereas stability was found higher at acidic pH range which indicates that *Cunninghamella* lipase can be exploited in the industries where

lipase activity over a broad pH range (4-10) is needed.

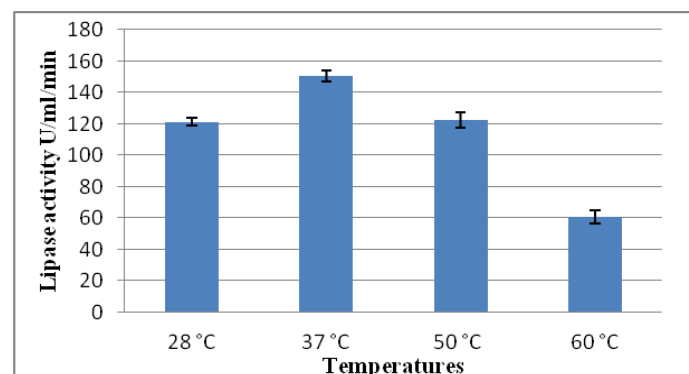


Figure-1: Determination of optimum temperature of *Cunninghamella* lipase.

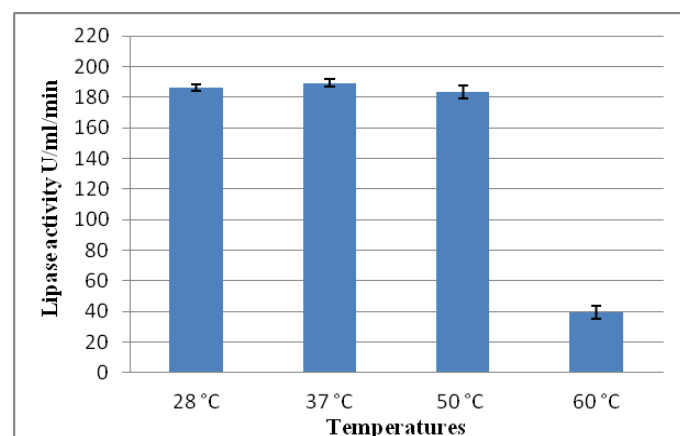


Figure 2: Determination of thermostability profile of *Cunninghamella* lipase.

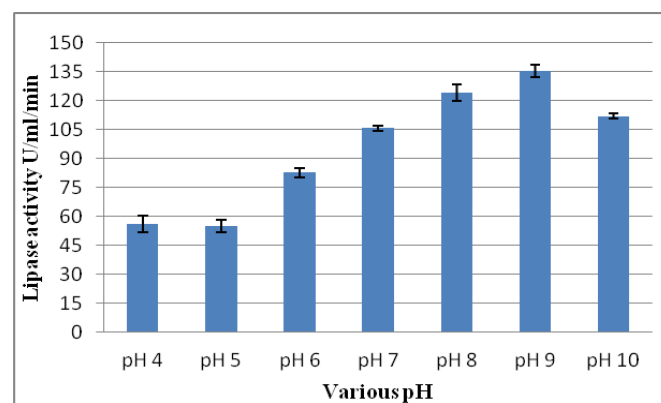


Figure 3: Determination of optimum pH of *Cunninghamella* lipase.

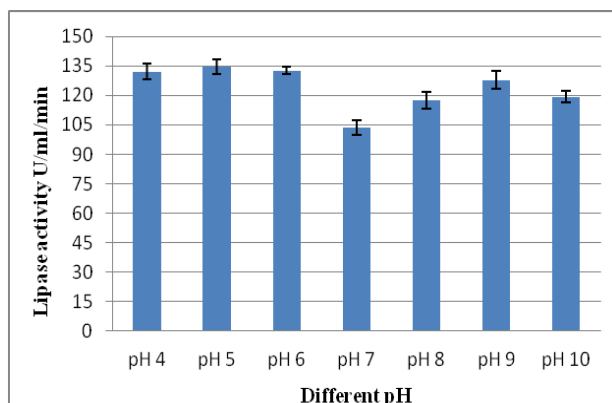


Figure 4: Effect of various pH on stability of *Cunninghamella* lipase

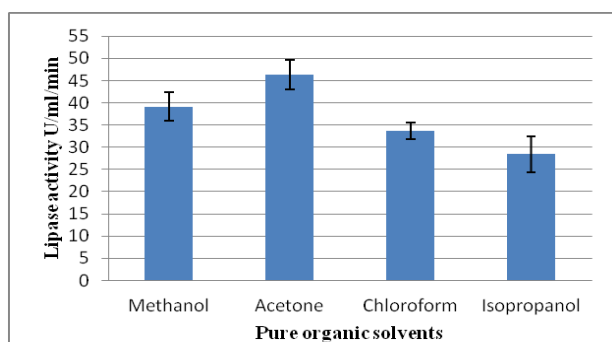


Figure 5: Effect of various organic solvents on activity of *Cunninghamella* lipase.

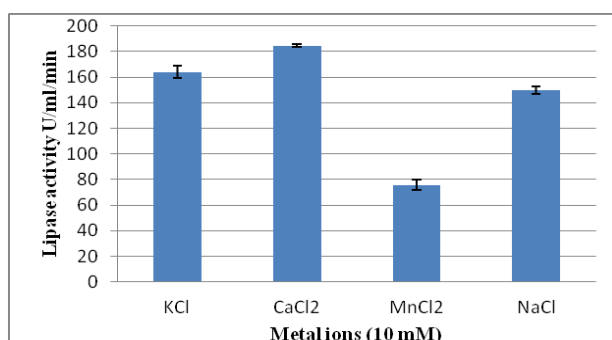


Figure 6: Effect of various metal ions on activity of *Cunninghamella* lipase.

In accordance with present findings, Gopinath et al. (2002) reported

highest activity of *Cunninghamella verticillata* lipase within the broad range of temperature (27 °C to 52 °C) and pH (5 to 9.5). Colla et al. (2015) reported that *Aspergillus* sp. lipase retained 80% of its activity in acidic pH range. An optimum pH of 4.6 was reported by Costa-Silva et al. (2014) for *Cercospora kikuchii* lipase. Zhou et al. (2012) reported maximum activity and stability of *Aspergillus oryzae* lipase at pH 4.0 thereafter activity and stability decreased with rise of pH and reached to minimum at pH 9.0. Very few reports are available about stability of lipase below pH 3.0, such acidic lipases are particularly useful in the food and flavor industries where reactions are performed in acidic environment (Hasan et al. 2006).

An optimum pH of 6.0 was reported for *Aspergillus terreus* lipase by Sethi et al. (2016) whereas Oliveira et al. (2014) reported optimum pH of 6.5 for *Candida guilliermondii* lipase. An optimum pH of 7.0 was reported by Sharma et al. (2016) for *A. niger* lipase and Mase et al. (1995) for *Fusarium* sp. lipase whereas optimum lipase activity at pH 7.5 was documented by Jayaprakash and Ebenezer (2012) for *Aspergillus japonicus* lipase and Gopinath et al. (2002) for *Cunninghamella verticillata* lipase.

Effect of organic solvents on lipase activity and stability

Enzyme exhibited maximum stability (46.32 ± 3.33 U/ml) when it was pre-incubated with acetone for 2 h. Lipase was also found stable when it was pre-incubated with methanol, chloroform and isopropanol. The descending order of lipase activity was as follows: acetone > methanol >

chloroform > isopropanol (Fig. 5). These results suggest that *Cunninghamella* lipase can be used in those industries where lipase catalyzed reactions (esterification and transesterification) are performed in non aqueous environments means in the presence of organic solvents.

Jayaprakash and Ebenezer (2012) investigated lipase activity of *Aspergillus japonicus* in presence of two different concentrations (10% and 20% v/v) of organic solvents. Significant residual lipase activity was obtained at 10% concentration of methanol (95%) and acetone (84%), activity was moderate with 10% concentration of butanol (65%) and chloroform (38%), 20% concentration of methanol (62%) and butanol (45%) and lowest activity was obtained with 10% concentration of hexane (5%) and ethanol (23%). Residual activity was completely lost with 20% concentration of acetone, chloroform and ethanol. Sharma et al. (2016) reported that methanol (10%, v/v) increased lipase activity of wild and mutant strains of *A. niger* up to 29% and 10%, respectively whereas lowest lipase activity was observed with butanol. Zhou et al. (2012) reported that glycerol (20% v/v) increased activity of *Aspergillus oryzae* lipase up to 10% whereas activity was completely lost with ethanol (20% and 30% v/v) and acetone (30% v/v).

Lipase activity in presence of metal ions

Among the all metal ions, calcium chloride was found to be best stimulator of lipase activity (184.69 ± 1.21 U/ml). Significant lipase activity was also seen when enzyme was pre-incubated with potassium chloride (163.95 ± 4.85 U/ml) and sodium chloride ($149.67 \pm$

3.11 U/ml). Lipase activity was lowest (75.85 ± 4.23 U/ml) with manganese chloride (Fig. 6). Optimum activity with calcium chloride might be due to that CaCl_2 interacted with enzyme, modified catalytic site and accelerated its activity. It can be concluded that CaCl_2 might be allosteric activator of *Cunninghamella* lipase.

Stimulatory effect of Ca^{2+} was reported by Gopinath et al. (2002) for *Cunninghamella verticillata* lipase. Costa-Silva et al. (2014) reported that divalent metal ions (Al^{3+} , Hg^{2+} , Ca^{2+} , Mg^{2+} , Zn^{2+} and Ba^{2+}) increased activity of *Cercospora kikuchii* lipase where as Na^+ , K^+ and Cu^{2+} decreased lipase activity. Activity of *Penicillium aurantiogriseum* lipase was enhanced by Zn^{2+} , Mn^{2+} and Mg^{2+} but it was suppressed by Hg^{2+} ion (Schmid and Verger, 1998). Oliveira et al. (2014) reported no change in the activity of *Candida guilliermondii* lipase in presence of K^+ , Zn^{2+} , Mg^{2+} and Mn^{2+} but it was increased up to 5% with Na^+ and declined up to 8% in presence of Fe^{3+} and 3% with Ca^{2+} . Sethi et al. (2016) reported strong inhibitory effect of Na^+ , Ag^+ and Hg^{2+} on activity of *Aspergillus terreus* lipase whereas Zn^{2+} , Mn^{2+} and Mg^{2+} exhibited stimulatory effect. Jayaprakash and Ebenezer (2012) documented that activity of *Aspergillus japonicus* lipase was completely lost in presence of Mn^{2+} and Co^{2+} , lowest activity with Cu^{2+} and Fe^{3+} , moderate activity with Na^+ and Ba^{2+} and significant activity was obtained with Ca^{2+} , Na^{3+} and Hg^{2+} . Calcium chloride was also reported as excellent inducer of lipase activity by Sharma et al. (2016) for *A. niger* lipase while Hg^{2+} decreased lipase activity drastically. Mase et al. (1995) stated that activity of *Fusarium*

sp. lipase was not influenced by Mn^{2+} , Ca^{2+} , K^+ , Mg^{2+} , Na^+ , and Cu^{2+} .

Conclusion:

Microbial lipases are an imperative group of biotechnologically precious enzymes, due to the versatility of their applied properties and simplicity of bulk production. Some basic information about properties of lipases is required before using them for industrial purposes. Therefore, the present investigation was aimed to partially characterize the extracellular lipase produced by submerged fermentation of *Cunninghamella* sp. Lipase possessed maximum activity at 37 °C and pH 9.0. Enzyme retained significant activity at 28 °C, 37 °C and 50 °C. In pH stability pattern, enzyme exhibited more stability in acidic pH range than in alkaline range. Significant lipase activity was obtained when enzyme was pre-incubated with acetone for 2 h. Among all metal ions, Ca^{2+} was found excellent inducer of lipolytic activity. The present results suggest that *Cunninghamella* lipase can be exploited in those industries where reactions are performed in broad range of temperature and pH.

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STANDARDIZATION OF THE OPTIMUM IRRIGATION, NUTRIENT MANAGEMENT PRACTICES IN CAPSICUM FOR PROTECTED CULTIVATION

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Abstract

The Present investigation was conducted during summer-rainy season, 2013 at Vegetable Research Block of Uttarakhand University of Horticulture and Forestry, Ranichauri Campus, Tehri-Garhwal in polyhouse equipped with thermo censored exhaust fans and drip irrigation system. The experiment was laid out in two factors RBD with five replications. The treatments in first factor included two methods of application of NPK viz., soil dressing (A₁) and drip fertigation (A₂) whereas second factor included four nutrient packages viz., N:P:K @ 160:80:40 kg/ha + FYM @ 20 t/ha (F₁), N: P: K @ 160:80:40 kg/ha + FYM @ 20 t/ha + lime @ 3.0 q/ha + PSB + *Azotobacter* (F₂), Observations were recorded on plant growth and fruit yield characteristics viz. number of fruits per plant, fruit yield per plant (g), The results indicated that the drip fertigation of NPK (A₂) appeared to be the most promising treatment in polyhouse grown capsicum for fruit yield (per plant as well as per m²) (870.0 g and (6.80 kg, respectively), number of fruits per plant (31.31), intervals starting from 45 days after transplanting would result in 990.0 g fruits per plant or 7.71 kg/m² area or 77.0 q/ha in temperate hills of Uttarakhand.

Key words: Capsicum plant, drip fertigation, soil dressing, nutrient packages, polyhouse

Introduction:

Capsicum (*Capsicum annum* L.) (Bell pepper, Sweet Pepper and Shimla Mirch) is a member of Solanaceae family. It is an annual herb that attains a height up to 1.0 meter. Capsicum was

brought to India from Brazil by Portuguese. Brazil is considered as its native place. Main shoot is radical but lateral branches are cinnate. One of the branches at each node remaining undeveloped and subtending bract or bracts are agnate and are carried up a

lateral shoot to node above. Leaves are variable in size, simple, petiole 0.5-2.5 cm long, lamina broadly lanceolate to ovate, entric, thin, sub-glabrous, 1.5-12.0 × 0.5-7.5cm, tip acuminate, base cuneate or acute. Flower usually borne singly and are terminal, but due to the form of branching, appear to be auxiliary; pedicels up to 1.5 cm long; clayxcanpanulate, shortly 5 dentate, 10-ribbed about 2 mm long enlarging and enclosing base of fruits; corolla, stamens 5-6 inserted near base of corolla, anthers bluish, dehiscing longitudinally; ovary 2-celled but after multiplying under domestication; style simple, white or purple; stigma capitate. Fruit indehiscent, many seeded berry, pendulous or erect borne singly at nodes, variable in size, shape, colour and degree of pungency, linear, conical or globose, 10-30 cm long, unripe fruit green or purplish, ripening to red or orange, yellow, brown, cream or purplish; seeds 3-5 mm long; pale yellow. Sweet peppers are very rich in vitamins, especially in A and C. The red bell peppers have different types of pigments. Capsanthin accounted for about 36% of the total carotenoid content β -carotene and violaxanthin for about 10% each, cryptocapsin and capsorubin about 6% each and cryptocapsin for about 4%. The vitamin C content was found as high as 321 mg/100g (Simon, 1960). Folic acid amounted to 1.3-2.9 mg/100g. Bell pepper content is 92.4%, water and the food value per 100g of edible portion energy is

29 calories, protein 1.2 g, calcium 11 mg, vitamin A 870 I.U, ascorbic acid 175 mg, thiamine 06 mg, riboflavin 0.03 mg, and niacin 0.55mg (Joshi and Singh, 1975). Due to its high nutritive value, it is often looked upon as luxury vegetable. It is considered to be cool season crop and well suited to different temperate regions and is presently grown in subtropical areas of India. Being a cool season vegetable crop, it is popularly grown in hills of Jammu & Kashmir, Himanchal Pradesh, Uttarakhand and Nilgiris during summer months. It is successfully raised in Maharashtra, Karnataka, parts of Tamil Nadu, Bihar, West Bengal, M.P and U.P. The area of capsicum in Uttarakhand hills is about 2,839 ha with an annual production of 11,921 metric tonnes. In India area under capsicum is 12,000 hectares with production and productivity of 1.60 lakh metric tonne and 137.1 q/ha, respectively (Anonymous, 2013). High demand of produce in nearby markets and metropolitan cities ensures the remunerative price to the growers of Uttarakhand hills.

The climatic conditions of Uttarakhand hills remain inconsistent particularly during summer-rainy seasons when capsicum and many vegetable crops are cultivated. Prevalence of prolonged low temperature below the optimum requirement and erratic rainfall necessitates, cultivation of high valued crops under controlled and protected

conditions is important for ensured and optimum yield. Inorganic fertilizer accompanied with combinations of lime, compost, bio-fertilizers and micronutrients used as solution or solid is essential to evaluate in capsicum for abstracting conclusions on INM and resource utilization efficiency under protected conditions.

Materials and Methods:

The experiment was conducted in polyhouse conditions at Uttarakhand University of Horticulture and Forestry Campus Ranichauri, Tehri-Garhwal (Uttarakhand). The details of the materials used and the procedure followed during this study have been described hereunder:

N:P:K @ 160:80:40 kg/ha + FYM @ 20 t/ha + lime @ 3.0 q/ha + PSB + *Azotobacter*.

Mode of application of Nutrients and Micronutrients:

1. Soil application:

NPK @160:80:40 kg/ha was applied through DAP (P = 46%, N = 18%), Urea (N = 46.4%) and Muriate of Potash (K = 60%). Zinc and Boron were applied @ 20 kg/ha of each through Zinc Sulphate (Zn = 33%, S = 15%), and Di Sodium Octaborate Tetra hydrate (Trade Brand *Folibor*, Coromandel International Ltd.) (B = 20%), respectively as soil dressing in standing crop during hoeing and earthing up.

2. Fertigation:

Above mentioned doses of N, P & K were applied in Capsicum crop with irrigation water through drip system. Foliar spray of commercial formulation containing B, Cu, Zn, Fe, & Pb @ 200 ppm was done thrice at fortnightly intervals invariably in both the methods of application of NPK. Fertigation was started one week of transplanting and applied once in a week for 20 minutes by dissolving 108 g of water soluble NPK + 47g DAP + 121g Urea in tank at the time of fertigation. Total numbers of 10 fertigation were in the whole growing season to supply NPK @ 1.08kg NPK (20:20:20), 0.47kg DAP and 1.21kg Urea for 54 m² area.

3. Irrigation:

All the plots under both the factors were irrigated through drip system at 4 days intervals for 20 minutes each time.

Observation taken:

1. Number of fruits per plant:

Number of fruits was counted at each harvest in the selected plants added and divided by number of selected plants to work out number of fruits per plant.

2. Fruit yield per plant (g):

Total weight of the fruits harvested from all the plants in each plot parts in all the harvests was recorded and summed up to get fruit yield per plot.

Results and Discussion:

Number of fruits per plant:

The data on number of fruits per plant indicated that there was a significant difference in application methods of NPK and nutrient combinations (Table 4.12). Maximum number of fruits per plant was recorded in the plots supplemented with NPK through drip (31.31) which was significantly superior to that in soil application of NPK (25.37). Among nutrient packages, F₄ exhibited highest number of fruits per plant (31.24) followed by F₃ (28.54) and F₂ (28.13) with non-significant difference. As far as interaction of application methods of NPK and nutrient package was concerned, drip fertigation of NPK + F₄ nutrient package was most superior combination for number of fruits per plant (35.34) followed by drip fertigation of NPK + F₃ (31.42) and drip fertigation of NPK + F₂ (30.91) which were statistically *at par*.

Corroborating the findings of this investigation, higher number fruits per plant in chilli have also been reported by Nateshet *al.* (2005) with foliar spray of 0.1% ZnSO₄ and application of vermin-compost, Balochet *al.* (2008) with foliar spray of formulation containing NPKCa and multi-micronutrients, Khan and Pariari (2012) with application of

Azospirillum and NPK and Pariari and Khan (2013) with application of vermin-compost + Urea.

Fruit yield per plant (g):

The data on fruit yield per plant depicted in Table indicated that there was significant difference among application methods of NPK and nutrient packages. Higher value of fruit yield per plant was found in plots supplemented with NPK as drip fertigation (870.0 g) as compared to that in soil application of NPK (510.0 g). The nutrient package F₄ was promising for fruit yield per plant (845.0 g), followed by F₃ (715.0 g). The interaction between application method of NPK and nutrient combination was also found significant in relation to this character. The treatment combination drip fertigation of NPK + F₄ was most suitable for increasing fruit yield per plant (990.0 g) followed by at par values in drip fertigation of NPK + F₃ (900.0 g) and drip fertigation of NPK + F₂ (860.0 g). Soil application of NPK did not appear so promising at any nutrient package. In this experiment, the advantage of application of water soluble NPK through drip fertigation could easily be realized on the basis of fruit yield per plant. The effectiveness of foliar application of micronutrients over only soil application could be noticed.

Table -1 Effect of application methods of NPK and nutrient combinations on Number of fruit per plant

Application Methods of NPK (A)	Nutrient Combinations (F)				Mean
	F ₁ (NPK @ 160:80:40 kg/ha + FYM @ 20 t/ha)	F ₂ (F ₁ + lime @ 3.0 q/ha + PSB + Azotobacter)	F ₃ (F ₂ + Boron @ 20 kg/ha + Zinc @ 20 kg/ha)	F ₄ (F ₂ + Boron @ 10 kg/ha + Zinc @ 10kg/ha + 3 foliar spray of multi micronutrients @ 200 PPM)	
Soil Application (A ₁)	23.32	25.35	25.66	27.13	25.37
Drip Fertigation (A ₂)	27.55	30.91	31.42	35.34	31.31
Mean	25.44	28.13	28.54	31.24	28.34
	CV (%)		SEM		CD (0.05)
(A)	13.14		1.33		3.85
(F)			1.88		5.4
(A×F)			2.66		7.7

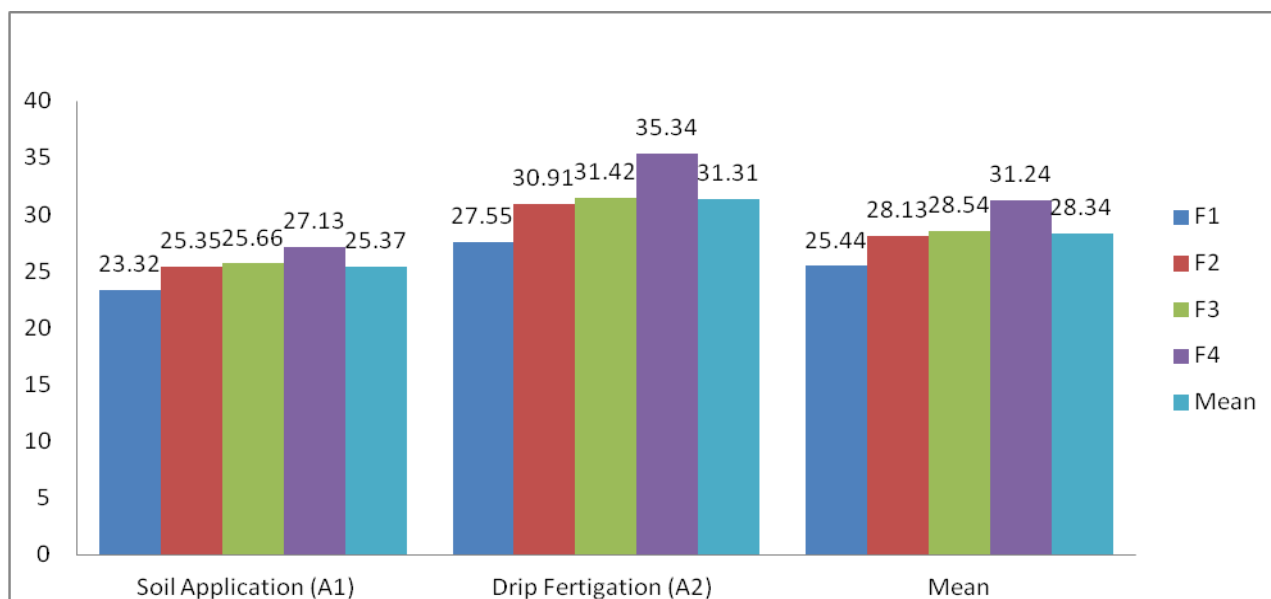


Fig. -1: Graphical presentation of Number of fruit per plant as affected by application methods of NPK and nutrient combinations on Number of fruit per plant.

Table -2 Effects of application methods of NPK and nutrient combinations on Fruit yield per plant (g)

Application Methods of NPK (A)	Nutrient Combinations (F)				Mean
	F ₁ (NPK @ 160:80:40 kg/ha + FYM @ 20 t/ha)	F ₂ (F ₁ + lime @ 3.0 q/ha + PSB + Azotobacter)	F ₃ (F ₂ + Boron @ 20 kg/ha + Zinc @ 20 kg/ha)	F ₄ (F ₂ + Boron @ 10 kg/ha + Zinc @ 10kg/ha + 3 foliar spray of multi micronutrients @ 200 PPM)	
Soil Application (A ₁)	350	450	530	700	510
Drip Fertigation (A ₂)	740	860	900	990	870
Mean	545	655	715	845	690.0
	CV (%)		SEM		CD (0.05)
(A)	12.24		34.0		90.0
(F)			48.0		140.0
(A×F)			69.0		200.0

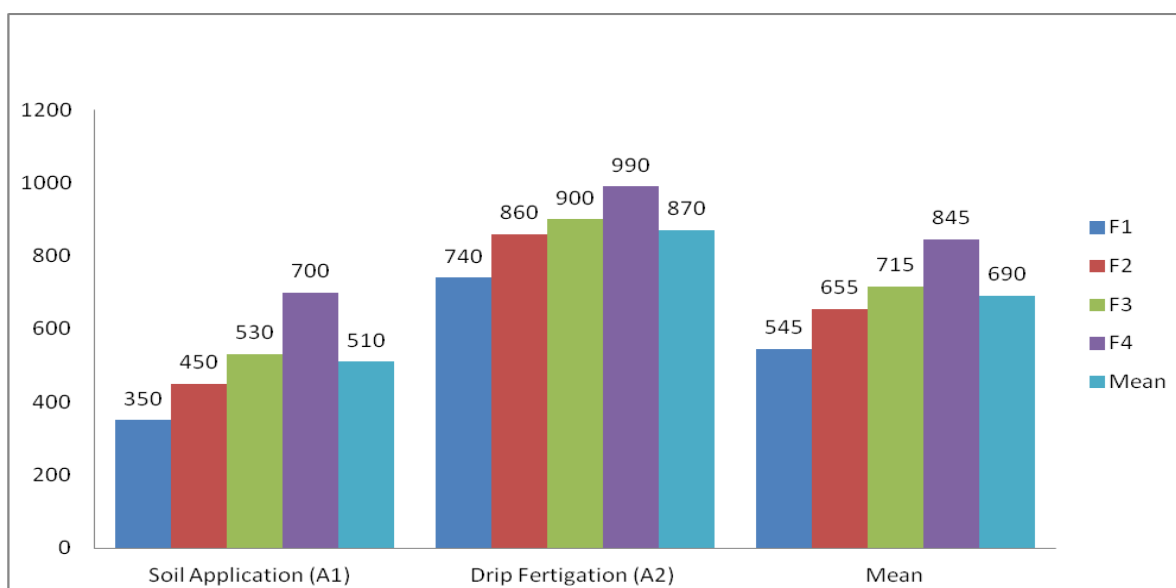


Fig.-2: Graphical presentation of Fruit yield per plant (g) as affected by application methods of NPK and nutrient combinations on Fruit yield per plant (g)

Conclusion:

The conclusion of present investigation revealed that the cultivation of capsicum in polyhouse conditions in temperate hill of Uttarakhand should adopt following operations:

1. Mixing of FYM @ 20 t/ha and lime @ 3.0 q/ha in the soil of poly house.

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By adopting above cultural operations the growers can harvest 990 g fruits per plant or 7.71 kg per square meter area or 771.0 q/ha in open pollinated cultivar of capsicum like PRC-1.

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EFFECT OF VARIOUS SEED TREATMENTS ON YIELD ATTRIBUTES OF POTATO CROP

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Abstract

Potato tubers of variety Kufri Jyoti and Atlantic (35 – 45 mm size) was taken as planting material. The effect of various seed treatment (Urea, Thio-urea and KMB) was examined by treated the seeds before planting. the observations of the study was number of stem per plant, number of tuber per plant and total yield, the haulm was killed at 75 days after planting and the data for number of tuber/plant and total yield were taken at 25 days after haulm killing at harvesting. It is observed that the seed treatment by urea at 5 % found most suitable concentration to increase the total yield of potato crop variety Kufri Jyoti in comparison to all other concentrations i.e. 1, 10, 15 % and in comparison to control also, but to establish the fact, the study must have recommended for further investigation in future. 1% thio-urea is also found most suitable concentration for early emergence, higher number of tuber per plant and highest yield of potato crop. It is also observed that with the increases in thio-urea concentration more than 1%, days taken for emergence as well as yield is also decreases. Seed treatment by KMB at 5 % also found most suitable concentration to increase the total yield of potato crop variety Kufri Jyoti in comparison to all other concentrations i.e. 1, 3, 7 % and in comparison to control also.

Key words: Potato, urea, thio-urea, KMB and seed treatment

Introduction:

The importance of potato (*Solanum tuberosum* L.) as one of the world's major staple crops is increasingly being recognized, because it produces more dry matter and protein per hectare than the major cereal crops. The nutritional value of potato tubers is a key factor for its progressive production, along with the economic benefits that potato cultivation can bring to developing

countries (Van Gijessel, 2005; McGregor, 2007). As a crop of high biological value for its protein and a substantial amount of vitamins, minerals and trace elements, potato is undoubtedly a very important crop in the country (Gebre and Sathyanarayana, 2001). Potato has the fourth rank among foods in terms of importance after wheat, rice and corn in the world (Germchi, *et al.* 2011). Higher yield and proper tuber size in terms of seed potato and ware potato is a very

important aspect to full fill the requirement of seed potato producers/farmers and for all the population of the country, because of increasing consumption of potato day by day. Potato is a global crop planted in a wider range of altitude, latitude, and climatic conditions. No other crop can match the potato in its production of food energy and food value per unit area (Davies et al., 2005). Nutrition analysis showed that potato is a healthy food in terms of vitamins, minerals, proteins, antioxidants, essential amino acids and carbohydrates (Andre et al., 2007). However, there are many problems surrounding potato cultivation. One problem is that potato plant has one of the heaviest production demands for fertilizer inputs of all vegetable crops.

The poor fertilizer management i.e. improper use of manures and fertilizers is one of the reasons for poor yield (Islam *et al.*, 1982). Nitrogen plays a major role in the production and maintenance of an optimum plant canopy for continuing tuber growth through long growing period (Westermann and Kleinkopf, 1985). Nitrogen fertilizer application is considered as one of the most important factor which limits production of potato (Tran and Giroux, 1991). Nitrogen has very low use efficiency and is lost easily due to which crop cannot use it and hence it increases economic concerns as crop production is less. Nitrogen which is not used by the crop is lost through leaching, runoff, volatilization and denitrification. This lost nitrogen increases contamination of water and gas emissions from greenhouse. If nitrogen losses are

reduced, crop nitrogen-use efficiency can be enhanced (Engelsjord *et al.*, 1997). The nitrogen losses can be minimized by using appropriate method of its application. Normal fertilizer application is around 1000 kg ha⁻¹ 10N-3P2O5-10K2O. N requirements are as high as 336 kg ha⁻¹ in traditional production system for an expected yield of 5000 kg ha⁻¹ (Davies et al., 2005; Lang et al., 1999). Current agriculture are facing increased cost of synthetic fertilizer, (agro) ecosystems desiccation caused by extensive use of water in crop production (Whitley and Davenport, 2003) and subsequent reduction in water supplies for irrigation, heightening publication about the environmental and healthy impact of biocide overuse (Lotter, 2003), and the nitrate leaching from overuse of fertilizers, therefore, a new program must be developed to address these challenges. Dormancy of potato tuber is defined as the physiological state in which autonomous sprout will not occur, even when the tuber is placed under ideal conditions for sprout growth (Rehman *et al.* 2001). Among the chemicals applied for breaking down the potato nodes dormancy, thio-urea, a catalyze inhibitor which tiggers potato tubers germination and healing tubers injuries especially when it is applied in an appropriate concentration (F. Mani *et al.* 2013). Also many studies reported that thio-urea treatment is not only more efficient to break dormancy but it increases also sprouts number, comparing to other chemicals like IAA and GA3 (Germchi *et al.* 2010). In addition, earlier workers also reported that thio-urea has great influence on yield and quality of potato

tubers (Panah *et al.* 2007), but the impact of thio-urea on plant growth and on quality of potato tubers is not well established. According to Rahman *et al.* 2003; Panah *et al.* 2007; Mani *et al.* 2011, treating tubers with thio-urea is efficient to break dormancy, but its impact on yield is not well established. Thereafter, applying beneficial microbial inoculants are emerging as a promising alternative for maintaining a sustainable agriculture system. Evidence shows that maintenance of sustainable soil fertility depends greatly on the ability to harness the benefits of plant-growth-promoting bacteria (PGPB) such as N-fixing, P-solubilizing bacteria (PSB), mycorrhizal helper bacteria (MHB), endophytes, and arbuscular mycorrhizal fungi (AMF) (Barea *et al.*, 2005; Smith and Read, 2008). The special focus on K solubilizer was due to the fact that potassium is one of the major nutrients required by all crops. It is a key element in many physiological and biochemical processes. Mineral potassium solubilization by microbes which enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble K (Rajan *et al.*, 1996). Potassium solubilizing bacteria are capable of solubilizing rock K, mineral powder such as mica, elite and orthoclases through production and excretion of organic acids (Fridrich *et al.*, 1991).

Current interest in the potassium fertility of soil has been changed from simple estimation of exchangeable K to measurement of the rate at which K is supplied from exchangeable fractions.

Rate of non exchangeable K release and its mechanism are controlled by nature and amount of clay minerals, besides this exploring the role of microbes present in the soil also started this exploring the role of microbes present in the soil also started recently. According to preliminary studies and crop response studies gives encouragement in this line (Chandra *et al.*, 2000, Chandra *et al.*, 2005). An interesting finding was made from Banana rhizosphere by Dr. Krishna Chandra during 1998 and noticed a microbe is predominant and play vital role in help plants in potassium nutrient uptake. Later it was authenticated by Institute of Microbial Technology (IMTECH), Chandigarh as *Frateuria aurentia* and known as Potash Mobilizing Bacteria (KMB), belonging to the family Pseudomonaceae. KMB is a beneficial free living soil bacteria isolated from rhizosphere of plants, which have been shown to improve plant health or increase yield are usually referred to as plant growth promoting rhizobacteria - PGPR (Kloepper *et al.*, 1980). A number of different nitrogen fixing and phosphate solubilizing bacteria may be considered to be PGPR including *Azotobacter*, *Azospirillum*, *Rhizobium* other bacterial genera e.g. *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas* etc. also reported as PGPR. According to Chandra *et al.*, 2005 and field trials, *Frateuria aurentia* also to be considered as PGPR. So, Seed treatment may be an important substitute for soil fertilization and a good tool in crop management to maximize yields of crops.

Materials and Methods:

Potato tubers of variety Kufri Jyoti and Atlantic (35 – 45 mm size) was taken as planting material. The effect of various seed treatment (Urea, Thio-urea and KMB) was examined by treated the seeds before planting.

In seed treatment potato tubers were wet by solution of Urea (1, 5, 10 and 15% concentration, indicated as T1, T2, T3 and T4 respectively) followed by control (T5 – without urea seed treatment), Thio-urea (1, 3, 5 and 7% concentration, indicated as T1, T2, T3 and T4 respectively) followed by control (T5 - without thio-urea seed treatment) KMB (1, 3, 5 and 7% concentration, indicated as T1, T2, T3 and T4 respectively) followed by control (T5 - without KMB seed treatment) done with spray method and were left to dried out till planting. Experiment was randomized block design with three replications for each concentration. Each concentration having 180 tubers in three replications, the observations of the study was number of stem per plant, number of tuber per plant and total yield, the haulm was killed at 75 days after planting and the data for number of tuber/plant and total yield were taken at 25 days after haulm killing at harvesting.

Tubers were planted in field at the spacing of 68.6 cm x 20 cm. The amount of fertilizers were used as 420 kg/Ha Urea, 350 Kg/Ha SSP, 250 Kg/Ha DAP, 300 Kg/Ha MOP and 30 Kg/Ha Zinc in whole cropping period. During the experiment period, each day show minimum of 5.9 °C and maximum of 24.6 °C air temperature. The soil temperature

was minimum of 10.3 °C and maximum of 22.3 °C.

Result and Discussion:

Effect of urea seed treatment on number of tuber per plant and total yield

The number of tuber per plant was observed at 25 days after haulm killing at harvesting (Table-1), the maximum number of tuber per plant were reported in T2 (7.00 ±0.60) followed by T3 (6.73 ±0.52), T1 (6.37 ±0.79), T4 (6.27 ±0.82) and T5 (5.03 ±0.43) concentrations. The yield was observed at 25 days after haulm killing at harvesting (Table-1), the highest yield (MT/Ha) were reported in T2 (21.20±3.54) followed by T5 (18.97±1.78), T1 (18.11±1.39), T3 (15.99±0.63) and T4 (15.85±3.70) concentrations. The present study indicates that the seed treatment with urea concentration shows positive influence on number of tuber and total yield in comparing to control. The maximum number of stem per plant was also found highest in T2 (4.27±0.27) concentration followed by T1 (3.53±0.13), T5 (3.47±0.13), T4 (3.33±0.07), and T3 (3.27± 0.24). Although specific review on the present study is not available but one study has been done by Behera, K.K. et al. (2009) on Greater Yam (*Dioscorea alata* L), In India *D. alata* tubers are consumed mainly in the southern and northern states. They are also cultivated as a cash crop in some area where they are more important than potato. In that study, they use vine cuttings as planting material and spread 1, 2 and 3% urea in nursery beds and an

another bed as untreated for control. From the experimental findings it is concluded that spraying of urea helps in rooting and increased the number of root

per cutting and there is an increase in the weight of tuber also, keeping this on view the present study has been investigated on potato crop.

TABLE 1: Effect of urea seed treatment on number of tuber per plant and total yield

S.N.	Urea Seed Treatment	No. of Stem/Plant	No. of Tuber/ Plant	Yield (MT/Ha)
1	T1	3.53±0.13	6.37±0.79	18.11±1.39
2	T2	4.27±0.27	7.00±0.60	21.50±3.54
3	T3	3.27±0.24	6.73±0.52	15.99±0.63
4	T4	3.33±0.07	6.27±0.82	15.85±3.70
5	T5	3.47±0.13	5.03±0.43	18.97±1.78
	C.D.	0.586	0.270	0.547
	SE(m)	0.177	0.602	2.582
	SE(d)	0.25	0.852	3.651
	C.V.	8.58	16.608	24.723

Effect of thio-urea seed treatment on number of tuber per plant and total yield

The number of tuber per plant was observed at 25 days after haulm killing at harvesting (Table-2), the maximum number of tuber per plant were reported in T1 (6 ±0.88) followed by T2 (6 ±0.57), T3 (6 ±0.00), T4 (5 ±0.33) and T5 (4 ±0.66) concentrations, this result is supported by Mani *et al.* 2013, who observed that using thio-urea the number of tuber per plant and dry matter of potato plants increased significantly. The yield was observed at 25 days after haulm killing at harvesting (Table-2), the highest yield were reported in T1 (16.1 ±1.45) followed by T2 (13.0 ±0.32), T5 (13.0 ±1.84), T3 (10.2 ±0.82) and T4 (8.0 ±0.47) concentrations. Present study shown that thio-urea concentration shows positive influence on number of tuber and total yield in comparing to control, similarly

Bajji *et al.* 2007, have also reported significant effect on tuber yield. It is also observed in the present study that number of tuber per plant and total yield (MT/Ha) was decrease with increases in thio-urea concentrations.

Effect of KMB seed treatment on number of tuber per plant and total yield

The number of tuber per plant was observed at 25 days after haulm killing at harvesting (Table-3), the maximum number of tuber per plant were reported in T3 (4.2 ±0.01) followed by T1 (3.6 ±0.21), T5 (3.3 ±0.23), T4 (3.2 ±0.03) and T2 (3.2 ±0.05) concentrations. The yield was observed at 25 days after haulm killing at harvesting (Table-3), the highest yield (MT/Ha) were reported in T3 (29.55±1.86) followed by T1 (25.47±5.85), T4 (24.94±1.84), T2 (24.07±3.74) and T5 (22.65±3.48)

concentrations. The present study indicates that the seed treatment of KMB concentration shows positive influence on number of tuber and total yield in comparing to control. The maximum number of stem per plant was also found highest in T3 (3.4 ± 0.40) concentration followed by T4 (3.3 ± 0.51), T2 (3.2 ± 0.40), T5 (3.2 ± 0.45), and T1 (3.0 ± 0.00).

Although the published information on the effect of KMB on potato crop and especially in the variety Kufri Jyoti is not available in the earlier literature but some other study may support the positive influence of KMB on growth and yield of a crop.

TABLE 2: Effect of thio-urea seed treatment on plant growth and yield of seed potato

S.N.	Thio-urea Seed Treatment	No. of Stem/Plant	No. of Tuber/Plant	Yield (MT/Ha)
1	T1	3.5 ± 0.13	6 ± 0.88	16.1 ± 1.45
2	T2	4.3 ± 0.26	6 ± 0.57	13.0 ± 0.32
3	T3	3.3 ± 0.24	6 ± 0.00	10.2 ± 0.82
4	T4	3.3 ± 0.06	5 ± 0.33	8.0 ± 0.47
5	T5	3.5 ± 0.13	4 ± 0.66	13.0 ± 1.84
	<i>C.D. at 5%</i>	0.576*	1.517*	2.889**
	<i>S.E. (m)</i>	0.177	0.465	0.887
	<i>S.E. (d)</i>	0.250	0.658	1.254
	<i>C.V.</i>	8.580	15.397	12.728

*Significant at 1%, **Significant at 5%

TABLE 3: Effect of KMB on number of tuber per plant and total yield

S.N.	KMB Seed Treatment	No. of Stem/Plant	No. of Tuber/Plant	Yield (MT/Ha)
1	T1	3.0 ± 0.00	3.6 ± 0.21	25.47 ± 5.85
2	T2	3.2 ± 0.40	3.2 ± 0.05	24.07 ± 3.74
3	T3	3.4 ± 0.40	4.2 ± 0.01	29.55 ± 1.86
4	T4	3.3 ± 0.51	3.2 ± 0.32	24.94 ± 1.84
5	T5	3.2 ± 0.45	3.3 ± 0.23	22.65 ± 3.48
	<i>C.D. @ 5%</i>	0.940	0.068	0.742
	<i>SE(m)</i>	0.387	0.228	3.687
	<i>SE(d)</i>	0.547	0.323	5.214
	<i>C.V.</i>	20.642	11.115	25.205

Microorganisms play a key role in the natural K cycle. Some species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. There are considerable population of Potassium Solubilizing Bacteria (KSB) in

soil and rhizosphere (Sperberg, 1958). Silicate bacteria were found to dissolve potassium, silicon and aluminium from insoluble minerals (Aleksandrov *et al.*, 1967). It has been reported that most of potassium in soil exists in the form of

silicate minerals. The potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch *et al.*, 1985) are the major soil groups in the state. In general, black soils are high, red soils medium and lateritic soils low in available K. Lateritic, shallow red and black soils have been found to show decline in K fertility over the years under intensive cultivation and imbalanced fertilizer application. Since K is a costly nutrient, India ranks 4th in consumption of potassium fertilizers. On an average 1.7 million tons of K is being imported annually (Anonymous, 2003). Currently, very little information is available on mineral potassium solubilization by bacteria, their mechanisms of solubilization and their effect on growth, K uptake and yield of several crops. Therefore the present investigation was undertaken to study the influence of potassium mobilizing bacteria on yield of potato crop.

Conclusion:

The conclusion of the present study is that seed treatment by urea at 5 % found most suitable concentration to increase the total yield of potato crop variety Kufri Jyoti in comparison to all other concentrations i.e. 1, 10, 15 % and in comparison to control also, but to establish the fact, the study must have recommended for further investigation in future. 1% thio-urea is also found most suitable concentration for early emergence, higher number of tuber per plant and highest yield of potato crop. It is also observed that with the increases in thio-urea concentration more than 1%, days taken for emergence as

well as yield is also decreases. Seed treatment by KMB at 5 % also found most suitable concentration to increase the total yield of potato crop variety Kufri Jyoti in comparison to all other concentrations i.e. 1, 3, 7 % and in comparison to control also.

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STUDIES ON CORRELATION AND PATH ANALYSIS IN FABA BEAN (*Vicia faba* L.) GENOTYPES IN HILL CONDITIONS OF UPPER HIMALAYA

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Abstract

The present investigation was carried out at the Research Block, Department of Crop Improvement, VCSG Uttarakhand University of Horticulture and Forestry, College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand. The experimental material comprised of 73 diverse faba bean genotypes. The crop of different genotypes was raised in an augmented design. Analysis of variance revealed among the checks were significant for six characters *viz.*, significant for days to maturity, field emergence, plant height (cm), number of pods per plant, pod length (cm) and seed yield per plant (g). The correlation revealed that seed yield per plant showed positive correlation with pod length, while days to maturity exhibited highly significant and positive correlation with days to 50% flowering, number of pods per plant showed significant positive correlation with field emergence and highly significant and positive correlation with plant height, number of seeds per pod showed significant and positive correlation with plant height and 100 seed weight also showed highly significant and positive correlation with number of pods per plant. The results of path coefficient analysis using simple correlation coefficient showed that highest positive direct contribution towards seed yield per plant was exhibited by pod length followed by number of pods per plant and days to maturity.

Key words: *Vicia faba*, Augmented design, correlation, path analysis

Introduction:

Faba bean (*Vicia faba* L.; $2n = 2x = 12, 14$) is a grain legume crop, which can be grown in tropical to temperate area. It is cultivated since ancient times and consumed as either green pod as vegetable or dry seeds as a pulse. It is also known as the broad bean, field bean, bell bean and tic bean. In India, it is popularly known as kala Matar and bakala (Singh *et al.*, 2013). Faba bean belongs to the family Fabaceae. Worldwide it is third most

important feed grain legume after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) in respect of area and production (Mihailovic *et al.*, 2005). This crop is quite useful in those areas where water is not found in excess quantity. This crop is also important due to the fixation of nitrogen to improve soil fertility. The area and productivity of faba bean is still very low in India due to unawareness and that is why it is still classified as underutilized crop. Faba Bean is grown in Jammu – Kashmir, Himanchal Pradesh, Uttarakhand,

Haryana and in the some part of South India in rainfed conditions (Singh and Bhatt 2012). In Uttarakhand state, Faba bean is grown in Uttarkashi, Tehri Garhwal, Udham Singh Nagar and Pauri district etc. The productivity of Faba bean in Uttarakhand state is quite lower as compared to other legume crops because of improper nutrient and pest management practices and also due to lack of suitable high yielding varieties for hills and plains of this state. In the hills of Uttarakhand, faba bean are grown during November to April. Poor adaptability to low temperature in rainfed condition at high altitudes and lack of high yielding cultivars are major constraints for increasing faba bean cultivation in hill regions of Uttarakhand. Moreover, the crop can be used as break crops where cereal based mono cropping system is dominated. The objective of study is to evaluate different germplasm of faba bean under high hills of Himalaya.

Materials and Methods:

The experimental site, College of Forestry of Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry, Ranichauri is located at 10 km away from Chamba (Rishikesh – Gangotri Highway) at an altitude of about 2100 m above mean sea level, lying between 30° 15' N latitude and 78° 30' E longitudes under mid hill zones of Uttarakhand, India. The field evaluations of the genotypes were carried out in the experimental block of Department of Crop Improvement. Ranichauri campus experiences humid and temperate types of climate with chilled winters. The mean monthly minimum and maximum temperature during faba bean crop period

(November to April) varies between 5.5 °C to 16.1 °C and 6.1°C to 16.8°C, respectively. The average annual rainfall of 1230 mm was experienced a last 20 years.

The 73 genotypes along with three checks (Vikrant, PRT 7 and PRT 12) were planted in an augmented design during *Rabi* -2013 under rainfed condition. There were 7 blocks, each block with 10 genotypes. The checks were allocated along with the new genotypes in block. Each block comprises of 10 genotypes, each genotypes sown in single row with three checks. Normal cultural practices were followed for growing the germplasm. The characters studied were Days to 50% flowering, Days to maturity, field emergence, Plant height (cm), Number of pods per plant, Pod length (cm), Number of seeds per pod, 100 seed weight (g), and Seed yield per plant (g). The analysis of variance for augmented design was done by using the method given by Federer (1956). The correlation between all characters under study was estimated as per the method described by Pearson (1956). The direct and indirect effects at genotypic level for genotypes were estimated by taking seed yield as dependent variable using, path analysis as suggested by Dewey and Lu (1959). Statistical software Windows version 9.2 was used for the analysis of data.

Results and Discussion:

The analysis of variance was carried out for all the characters and result are presented in Table 1. The difference among the entries were significant for days to maturity, field emergence, plant height (cm), number of pods per plant (cm), pod length (cm) and seed yield per plant (g)

and remaining were found non-significant. The differences among the checks were significant for six characters viz., days to maturity, field emergence, plant height (cm), number of pods per plant, pod length (cm) and seed yield per plant (g) and days to 50 % flowering, number of seeds per pod and 100- seed weight (g) were found non-significant. Simple correlation coefficient was computed among the 9 characters. Seed yield per plant showed positive correlation with pod length (0.057). Thus pod length emerged as most important factor influencing seed yield in faba bean. Days to maturity exhibited highly significant and positive correlation with days to 50% flowering

(0.409***). Number of pods per plant showed significant positive correlation with field emergence (0.243*) and highly significant and positive correlation with plant height (0.475***). Number of seeds per pod showed significant and positive correlation with plant height (0.248*). 100- seed weight also showed highly significant and positive correlation with no. of pod per plant (0.482**). While, during this study, the seed yield was negatively correlated with days to 50% flowering (-0.194), days to maturity (-0.042), field emergence (-0.017), plant height (-0.109), number of pods per plant (-0.052), number of seeds per pods (-0.115) and 100 seed weight (-0.192).

Table-1: Analysis of variance (ANOVA) for different characters of faba bean genotypes

S.N.	Characters df	Mean of squares					
		Block	Entries	Checks	Varieties	Checks vs. Varieties	Error
		6	72	2	69	1	12
1.	Days to 50% flowering	35.54	20.38	13.76	20.28	40.37	31.70
2.	Days to maturity	33.63	54.42*	128.04*	45.34	533.64**	19.15
3.	Field emergence	30.67	40.84**	66.84*	40.25**	29.56	10.81
4.	Plant height (cm)	5.94	30.58**	20.17**	31.28**	3.02	2.79
5.	Number of pods per plant	7.09*	5.81*	7.29	5.81*	2.74	2.08
6.	Pod length (cm)	0.09	0.43*	1.32**	0.36	3.53**	0.18
7.	Number of seeds per pod	0.11	0.25	0.63	0.23	1.02*	0.21
8.	100 seed weight (g)	46.40	17.75	40.29	17.06	20.13	23.79
9.	Seed yield/plant (g)	1.67	3.23*	14.25**	2.87	5.81	1.24

* Significant at 0.05 % level; **Significant at 0.01 % level

It is due to ability of yield primary components to compensate each other and therefore an improvement in one of them will lead to decrease in the other

components. It also indicated that these characters are of low predictive value to seed yield per plant. This literature also reported by Ahmed *et al.*, (2013).

Table - 2: Estimation of simple correlation coefficients between different characters in faba bean.

S. No.	Characters	Days to 50% flowering	Days to maturity	Field emergence	Plant height (cm)	Number of pods per plant	Pod length (cm)	Number of seeds per pod	100 seed weight (g)	Seed yield/plant (g)
1	Days to 50% flowering	1.00000	0.40910* **	-0.00016	-0.18452	-0.19037	0.15659	-0.03232	-0.02494	-0.19442
2	Days to maturity		1.00000	0.08827	-0.16166	-0.01290	-0.00675	-0.02665	0.01357	-0.04287
3	Field emergence			1.0000	0.05434	0.24383*	0.12118	-0.10976	0.14375	-0.01774
4	Plant height (cm)				1.00000	0.47555* *	-0.00764	0.24801*	0.17247	-0.10918
5	Number of pods per plant					1.00000	-0.11526	0.17626	0.48200***	-0.05208
6	Pod length (cm)						1.00000	0.14425	0.14943	0.05716
7	Number of seeds per pod							1.00000	0.46804***	-0.11522
8	100 seed weight (g)								1.00000	-0.19286
9	Seed yield/plant (g)									1.00000

Significant Levels 0.05 0.01 0.005 0.001
If correlation $r >$ 0.23027 0.29968 0.32517 0.37729

Table-3: Estimation of path coefficient analysis for different characters in faba bean.

S.N.	Characters	Days to 50% flowering	Days to maturity	Field emergence	Plant height (cm)	Number of pods per plant	Pod length (cm)	Number of seeds per pod	100 seed weight (g)	Seed yield/plant (g)
1	Days to 50% flowering	-0.2475	-0.1013	0.0000	0.0457	0.0471	-0.0388	0.0080	0.0062	-0.1944
2	Days to maturity	0.0167	0.0409	0.0036	-0.0066	-0.0005	-0.0003	-0.0011	0.0006	-0.0429
3	Field emergence	0.0000	-0.0024	-0.0272	-0.0015	-0.0066	-0.0033	0.0030	-0.0039	-0.0177
4	Plant height (cm)	0.0289	0.0253	-0.0085	0.1565	-0.0744	0.0012	-0.0388	-0.0270	-0.1092
5	Number of pods per plant	-0.0226	-0.0015	0.0289	0.0564	0.1186	-0.0137	0.0209	0.0572	-0.0521
6	Pod length (cm)	0.0235	-0.0010	0.0182	-0.0011	-0.0173	0.1503	0.0217	0.0225	0.0572
7	Number of seeds per pod	0.0005	0.0004	0.0018	-0.0040	-0.0029	-0.0023	-0.0162	-0.0076	-0.1152
8	100 seed weight (g)	0.0060	-0.0033	-0.0346	-0.0415	-0.1160	-0.0360	-0.1127	-0.2407	-0.1929

R square = 0.1146

Badolay *et al.* (2009), Osman *et al.* (2013) and Verma *et al.* (2013) reported significant positive correlation between seed yield and number of pods per plant, pods per plant with seed yield per plant, seed yield per plant with number of branches per plant, number of pods per plant, number of seeds per pod, biological yield per plant and harvest index. So they told that these characters could be emerged as most important factors influencing seed yield in faba bean. These observations were also reported by Alan and Geren (2007), and Ahmed *et al.* (2008). Hence these characters can emerge the most important factors influencing seed yield in faba bean.

The results of path coefficient analysis using simple correlation coefficient among nine characters are given in Table 3. The highest positive direct contribution towards seed yield per plant was exhibited by pod length (0.150) followed by number of pods per plant (0.118) and days to maturity (0.040). The days to 50% flowering (-0.247), field emergence (-0.027), plant height (-0.156), number of seeds per pod (-0.016) and 100- seed weight (-0.240) were negative direct effect on seed yield per plant. These characters have also identified as major direct contribution of yield by Peksen (2007), Bora *et al.* (1998), Salem (1982).

Conclusion:

So, Based on results of correlation coefficients, it may be concluded that number of Seed yield per plant showed positive correlation with pod length. Thus pod length emerged as most important factor influencing seed yield in faba bean, whereas, Days to maturity, Number of pods per plant,

Number of seeds per pod, 100- seed weight were other most important characters to be considered during selection for improving seed yield per plant in temperate hills of Uttarakhand. Path analysis can be useful as a technique to know the direct and indirect contributed effect of different characters to seed yield in crop so that the correlated importance of various yield contributing characters can be assessed. So, the above discussion suggests that the pod length, no. of pod per plant and days to maturity are the most important factors responsible for seed yield. So, the characters mentioned above should be given due consideration at the time of selection for high yielding varieties in faba bean.

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EVALUATION OF SUITABLE PLANT EXTRACTS FOR RETAINING STORAGE QUALITY OF APPLE THROUGH POST HARVEST APPLICATION

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Abstract

The experiment was laid out in a Factorial Completely Randomized Design (FCRD) with three replications and 5 treatments namely; T₁ (bael leaves extract @ 15%), T₂ (bael leaves extract @ 25%), T₃ (turmeric powder @ 15%), T₄ (turmeric powder @ 25%), and T₅ (control). After harvesting of fruits were treated with different botanical extracts treatments and stored in room temperature from 0 days to 120 days and physico-chemical analysis was done at an interval of 30 days viz., initial day, 30 days of storage, 60 days of storage, 90 days of storage and 120 days of storage. The present investigation clearly revealed that post harvest treatments of apple fruits with different botanical extracts was effective in increasing the shelf life of fruit as it helps in decreasing the PLW and rotting percentage of fruits and also helps in increasing the physico-chemical characteristics of the fruits. Among the treatments highest (7.36%) PLW and decay rotting (45.55%) was observed in T₅ (control) and lowest (3.09%) PLW was recorded in T₁ (bael leaves extracts @ 15%). After 120 days of storage maximum total sugar (11.76%) were observed in T₃ (turmeric leaves extract @ 15%) and maximum reducing sugar (9.90) was found in T₅ treatment (control) was at par with the treatments having lowest physiological loss in weight, rotting percent and highest total sugars.

Key Words: Apple fruit, post harvest and plant extract

Introduction:

Apple (*Malus domestica*. Borkh) belongs to family Rosaceae, is the premier fruit of the world. It is originated in Eastern Europe and Western Asia in the Caucasus region near Gilan and domesticated by Greeks and Romans a few centuries BC in the Middle-

East and South-Eastern Europe as a result of travels invasions (Shoemaker and Teskey, 1959). The movement of apple to Western Europe was helped by the Christian settlers. Romans are believed to have been responsible for its introduction in France and England. It has been cultivated in Europe for over 2,000 years and was brought to North America by the

earlier settlers in early seventeen century. There are still natural forests of apple on the Caucasus mountains of Southern Asia. In India it is cultivated in an area of 0.31 mHA with 1.915 mMT production and 6.1 MT/ HA productivity (NHB, 2013). The leading producing state is Jammu and Kashmir contributing 70.4% of total production followed by Himanchal Pradesh (21.5%), Uttarakhand (6.4%) and Arunachal Pradesh (1.6%), Apple was first introduced in Uttarakhand by F.E. Wilson in 1859 at Garhwal areas (Harsil areas of Uttarkashi District) while it is introduced in Kumaun hills by Mr. Allen and Mr. Smith at Chaubatia around 1872 (Chaddha and Awasthi, 2005). In 2013 the area under this crop in Uttarakhand was 0.037 mHA from which 0.13 mMT is produced with the productivity of 3.7 MT/HA (NHB, 2013). Nutritionally an apple having average fruit weight (182g) containing following nutrition carbohydrate 25g, sugar 19g, vitamin C 11mg, 150 calorie, iron 1mg, calcium 13mg, protein 1g (USDA 2012). The desired qualities in the modern commercial apple are colourful skin, absence of russetting, ease of shipping, lengthy storage ability, high yield and disease resistance (Anon, 2010). Higher plants contain a wide spectrum of secondary metabolites such as phenols, flavanoids, quinones, tannins, essential oils, alkaloids, saponins and sterols. Such plant-derived chemicals may be exploited for their different biological properties (Wain 1977; Tripathi and Dubey 2004). Biologicals because of their natural origin are biodegradable and mostly do not leave toxic residues or by products.

Although the neem based formulation are available in the market but there are other plants also which are having growth regulating and fungicidal properties like *Melia*, *Mentha* etc. (Grainge *et al.*, 1984). Similarly, the leaf extract of bael showed antifungal activity (Ganguly, 1994).

For the most part, the post-harvest practices followed by Indian growers and contractors are poor as compared with those followed in the United States and other major apple producing countries. In the past, incentives to improve post harvest practices were weak, likely because of the limited domestic market for higher quality and higher priced products, as well as the price risk faced by growers and contractors. Extension of shelf life is attributed to varietal characteristics, maturity, pre-cooling, storage temperature, relative humidity, and accumulation of carbon dioxide, ethylene and other gases in the storage atmosphere. The very objective for shelf life extension in apple can be achieved to some extent by using storage in modified packaging, perforated polythene, fungicides and chemicals etc and thus modified atmosphere packaging can be used to extend the shelf life of apple fruits (Lakakul *et al.*, 1999). It is essential to reduce both quantitative and qualitative losses by adopting suitable pre and post harvest handling procedures. With the ever increasing demand for good quality fruits, which are free from fungicide pesticide residues, the growers are forced to produce quality fruits, especially after the removal of restrictions on international trade

Materials and Methods:

The present investigation entitled evaluation of suitable plant extracts for retaining storage quality of apple through post harvest application was carried out in post-harvest laboratory, Department of Horticulture, College of Forestry (Uttarakhand University of Horticulture and Forestry) Ranichauri Tehri Garhwal, Uttarakhand, India. The details of the materials used and the procedure followed during this study have been described hereunder:

T₁ *Aegle marmelos*. (bael leaves extract @ 15%)

T₂ *Aegle marmelos*. (bael leaves extract @ 25%)

T₃ *Curcuma longa*. (turmeric powder @ 15%)

T₄ *Curcuma longa*. (turmeric powder @ 25%)

T₅ Water washes (control)

The preparation of coating solution aqueous extracts of different plant materials was prepared under laboratory condition on per cent weight basis as per the method described by Gakhukar (1996) and Sharma *et al.* (1997). Procedures adopted for recording observations on the various parameters for fruit weight, fruit length, fruit diameter, fruit volume, fruit firmness. Physiological loss in weight, Total Sugars.

Results and Discussion:

The data presented in Table 1 shows that PLW is significantly varies among different treatments and storage intervals. Among the treatments highest PLW (7.36%) was recorded in T₅ (control) and lowest (3.09%) in T₁ (bael leaves extract @ 15%) which was at par with T₂ (3.56%), T₃ (4.18%). While at different storage intervals PLW percent increases ranging from 2.09% to 13.00 % from 0 days to 120 days of storage. The data also predicts that there was significant difference in treatment among the interactions. Minimum PLW was observed in (1.02 %) in T₃ (turmeric powder @ 15%) It was observed PLW increases with increase in storage duration under all treatments. It is a well known fact that with an increase in storage duration the respiratory and transpiratory losses keeps on increasing which result in loss of metabolites and moisture ultimately resulting in lower fruit weight (Wikinson, 1965; Wills *et al.*, 1980; Singh and Rana, 1992). The physiological loss in weight was significantly reduced by bael leaf extract treatment. This may be due to the fact that bael leaf extract check the growth of microbes that are responsible for higher metabolic rate, which cause loss in weight through transpiration. Effectiveness of plant leaf extracts in reducing the physiological loss in weight and prolonging the shelf life of fruits in comparison to control as found in present study can be corroborated by previous

Table - 1 Effect of botanical extracts on decay/rotting (%) of apple fruits during storage.

TREATMENTS	STORAGE DAYS				MEAN
	D2(30)	D3(60)	D4(90)	D5(120)	
T 1	4.42	24.44	39.99	53.33	30.54
T 2	11.10	26.66	42.22	59.99	34.99
T 3	4.44	17.77	37.77	46.66	26.66
T 4	11.10	22.20	35.55	53.33	30.54
T 5	17.77	33.33	53.33	77.77	45.55
MEAN	8.84	24.43	41.49	58.51	
	Treatment (T)		Storage Days(D)		Interactions (T×D)
SE±	1.36		0.78		2.72
CD _{0.05}	3.82		2.20		7.64

Table – 2 Effect of botanical extracts on total sugar (%) of apple fruits during storage. Correct.

TREATMENTS	STORAGE DAYS					MEAN
	D1	D2	D3	D4	D5	
T 1	9.83	10.45	11.88	12.42	13.9	11.70
T 2	9.83	10.85	11.44	12.38	13.92	
T 3	9.83	10.46	11.65	12.62	14.25	11.68
T 4	9.83	10.44	11.67	12.47	14.06	11.76
T 5	9.83	10.24	11.26	12.05	13.56	11.69
MEAN						11.39
	9.83	10.43	11.57	12.42	13.95	
	Treatments (T)		Storage Days(D)		Interactions (T×D)	
SE±	0.97		0.56		0.19	
CD _{0.05}	0.27		0.15		0.54	

Table – 3 Effect of botanical extracts on sensory evaluation (flavour) of apple fruits during storage.

TREATMENTS	STORAGE DAYS				
	D2(30)	D3(60)	D4(90)	D5(120)	MEAN
T 1	7.38	7.43	6.42	5.44	6.67
T 2	7.41	6.86	5.81	5.21	6.32
T 3	7.42	7.26	6.40	5.60	6.67
T 4	8.15	7.22	6.46	6.27	7.02
T 5	6.77	6.53	5.28	3.55	5.53
MEAN	7.70	7.11	6.27	5.46	
Treatments (T)		Storage Days(D)		Interactions (T×D)	
SE±	0.20		0.12		0.41
CD _{0.05}	0.58		0.33		1.17

findings in various fruits, like mango, guava and banana (Khanna and Chandra, 1989; Purohit, 2000; Singh *et al.*, 1993 and Singh *et al.*, 2000).

Total sugar

The critical examination of data indicates that total sugar content varied significantly among all the treatments and storage intervals in (Table 2) Post harvest applications of almost all the botanical extracts were effective in increasing the sugar content of the fruit in comparison to the control except the treatment of mint leaves extract @ 15%. Among the treatments maximum total sugar (11.76 %) was recorded in T₃ treatment

(Turmeric @ 15%) and minimum (11.39%) in T₁₂ treatment (control). While at different storage intervals total sugar ranging from 9.83 to 13.95 percent from 0 days to 120 days storage. The data also shows that there was significant difference in treatments among the interactions. Maximum total sugar (14.25 %) was observed in T₃ treatment (Turmeric @ 15%) at 120 days storage while minimum (9.83 %) after the harvesting of fruits (initial period).

The apple fruit accumulate starch at the early stages of maturation that is later on hydrolyzed to sugars at edible maturity (Margein and Leurquin, 2000). The starch to sugars conversion continue

during storage (Beaudry *et al.*, 1989), resulting in increased total sugars with storage duration (Crouch, 2003). The increase in sugars during storage is therefore in line with the observation on loss of starch during the storage period. Chauhan and Joshi (1990) reported the efficacy of phytoextracts on the storage quality of mango cv. Ratna and found them significantly better in retaining total soluble solids and sugar content of the fruit. Treatment of *Citrus sinensis* fruit with neem formulations prior to harvest have also been reported to retain higher total soluble solids and total sugar contents (Bhardwaj and Sen, 2003).

Flavour

The data presented in (Table 3) and found that mean sensory scores for fruit flavour varied from 7.02 to 5.53 among different treatment and it was observed that highest score (7.02) was noticed in fruit treated with T₄ (turmeric @ 25%) which was at par with almost all the treatments of plant extracts except with T₂ (Bael leaves extract @ 25%) while minimum (5.53) was noticed in T₁₂ (control).

It was also found that during storage days sensory scores for fruit flavour show the declining trend from 7.70 to 5.46 after 0 days to 120 days of storage similarly among different treatment combination the declining trend for fruit flavor was noticed ranging from T₂ (Bael leaves extract @ 25%) at 120 days.

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