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HISTOPATHOLOGICAL EFFECTS OF PROCAMALLANUS INFECTION ON KIDNEY, INTESTINE AND LIVER OF CLARIAS BATRACHUS

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ABSTRACT

Experimental effects of *Procamallanus* infection on kidney, intestine and liver of *Clarias batrachus* were demonstrated 15th, 30th, 45th and 60th day of post infection. Liver of the infected fish showed infiltration of lymphocytes, mild dilation in central vein with distinct necrotic zone around central vein. Hepatocytes also revealed some degenerative changes. Fibrotic layer alongwith focal collection of lymphocytes and pyknotic nuclei was observed around the portal tract. Pyknosis, karyolysis, edema and cloudy swelling was also observed. Intestine of the infected catfish showed separation of serosa and sub-mucosa, ruptured serosa, strong inflammatory edema in villous processes. In kidney, rupture of Bowman's capsule wall, proliferation & shrinkage in glomeruli, necrosis, pyknosis, karyolysis, karyorrhexis in PCT.

Cloudy swelling, edema and inflammatory lymphocytes, renal tissue of infected fish showed numerous glomeruli or variable sizes were observed, the histopathological changes observed in kidney, liver and intestine of the *Clarias batrachus* due to the infection of *Procamallanus* appeared to be induced by excretory or secretory metabolites produced by the parasite.

Keywords **:** *Procamallanus, clarias batracus*, histopatological, catfish.

INTRODUCTION

Procamallanus is a nematode parasite commonly found in the intestine of fresh water such as *Clarias batrachus* and *Heteropneustes fossilis*, marine water and brackish water fishes. The infection caused by the helminths in the fish result in the deterioration in food value of fish and high mortality rate.

The high parasitic worm infection in the fish can lead to the reduction in their population size as it reduces the reproductive potential or delay sexual maturity. A cloudy swelling, fibrosis and degeneration in the kidney was observed in the *Clariasma crocephalus* whose stomach and intestine has the occurrence of *Procamallanus planolatus*. This is due to the reason that parasites

secrete or excrete endotoxins in the circulatory system of the host that further affects other tissues or system of the body including changes in the enzymes, vitamins, blood or hormonal activities.

MATERIALS AND METHODS

The freshwater catfish (*Clarias batrachus*) was collected from the local freshwater ponds, some fishes were purchased from the fish markets of Meerut and some other regions of Western Uttar Pradesh.

Before starting the experiment, these fishes were acclimatized for a week under laboratory conditions. The adult female *Procamallanus* were collected from the intestines of the infected catfishes. They were stored in the watch glass with saline solution at 24-27° C for natural egg laying. For healthy embryonation, these eggs were kept on the Lock-Lewis solution. O.1% of formalin was added to this solution to avoid fungal contamination and this solution was changed periodically. The experimental infection with 500 embryonated eggs of the nematode was induced in the healthy catfish.

The control and infected experimental fishes were sacrificed on 15th, 30th, 45th and 60th day and liver, kidney and intestine were removed surgically and fixed in the freshly made Bouin's solution. These tissues were then dehydrated in ascending series of alcohol and then cleared with xylene. These tissues were then embedded in paraffin wax at 60°C. At last sections of 6 µm were cut serially and stained with the help of haematoxylin and eosin for the histopathological examination.

RESULTS AND DISCUSSION

Intestine

Normally, the intestine of the *Clarias batrachus* is lined with a folded epithelium in the form of villi. These villi are taller and bigger in the anterior region. At some places these villi are numerous and fused together. Mucosa layer consisting of absorptive and mucosa secreting cells is composed of columnar epithelium cells. On the other hand, sub-mucosa was vascular and was extending into the villi as lamina propria. Intestine's muscular layer was made up of outer longitudinal muscle fibres and inner circular muscles. Intestine's anterior part is mainly secretory while posterior and lower epithelium are absorptive in nature.

After 15 days of *Procamallanus* infection, the intestine of the catfish shows separation of serosa and sub-mucosa, vacuolization in muscle layer and sub-mucosa, pyknotic epithelial cells in mucosa, ruptured serosa, strong inflammatory edema in villous processes and separation in muscle layer.

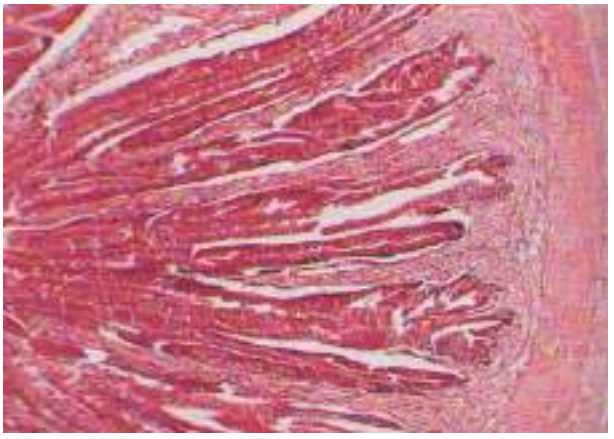

Fig: 1 T.S Passing through the intestine of control group showing normal structure of Serosa, longitudinal muscle layer, circular muscle layer, sub-mucosa, muscularis mucosa and mucosa after 15 days

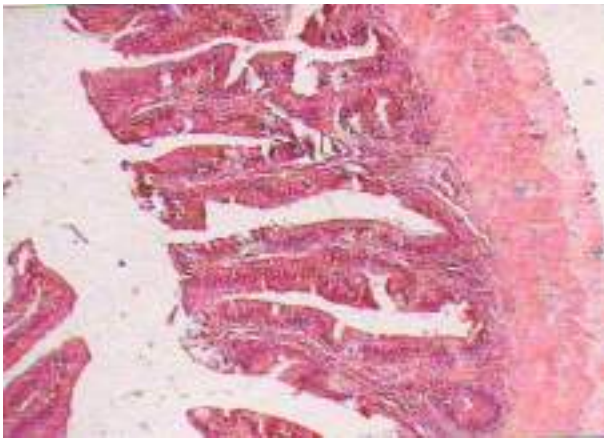

Fig: 2 T.S Passing through the intestine infected with dose of 500 embryonated eggs showing vacoulisation in sub-mucosa , ruptured serosa, strong inflammatory edema in vellous process and blunted tips of villi after 15 days.

On 30th day after infection, histopathological changes were seen like ruptured serosa, breakage and multiple fusions of villous processes, ruptures and vacuolization in serosa, blunted villi, inflammatory edema in longitudinal muscle layer and serosa, edema in longitudinal muscle layer and serosa.

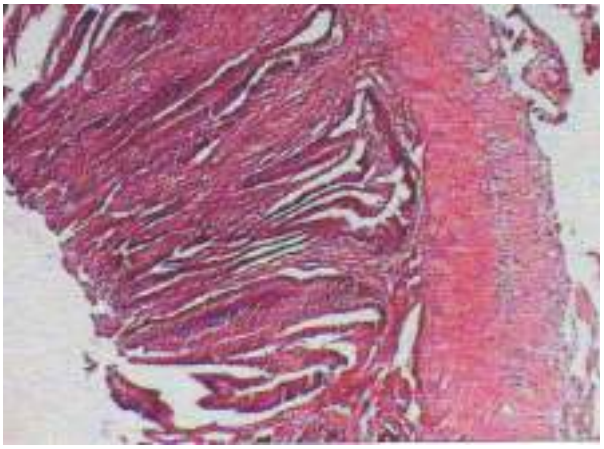

Fig: 3 T.S Passing through the intestine infected with dose of 500 embryonated eggs showing ruptured serosa, strong inflammatory edema in longitudinal muscle layer and serosa and fusion of villous process after 30 days.

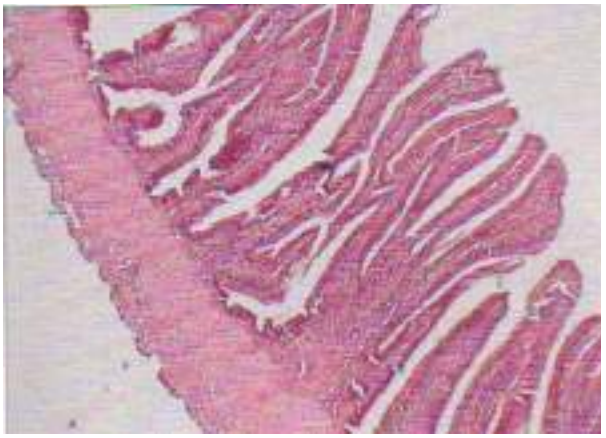

Fig: 4 T.S Passing through the intestine infected with dose of 500 embryonated eggs showing ruptured and vacuolization in serosa, blunted villi and edema in longitudinal muscle layer and breakage in villous process after 30 days.

After 45 days of infection, the pathological changes that were seen are edema, separation of muscle layer from peritoneal epithelium, blunted, breakage and separation of villous processes with large space, dilation of blood vessels, strong inflammatory edema and vacuolization in circular muscle layer and sub-mucosa.

60 days' post infection, these changes were adverse along with some other changes like shortened and truncated villi, vacuolisation in peritoneum layer and strong inflammatory edema.

Liver

Peritoneal layer of mesothelium covers the surface of the liver of *Clarias batrachus*. The peritoneal layer is underlined by dense connective tissue layer. Liver of untreated catfish consisted of polygonal hepatocytes, centrally placed nucleus and granular cytoplasm. Between these hepatocytes, connective tissue networks divide them chords. In the liver parenchyma, sinusoids are arranged irregularly. Liver is also supplied by blood vessels surrounded by haemopoietic tissue. Between the two layers of cells run the bile canaliculi that formed a network of ducts draining into a canal herring. This canal of herring entered the portal canal and merged with fine branches of the bile duct.

The portal tract present here is formed of portal vein, hepatic artery, and the bile duct. Endothelial cells with distinct nuclei form the wall of sinusoids. Throughout the connective tissue of liver, few elastic fires were found.

On 15th day of infection, a mild dilation in central vein and infiltration of lymphocytes was observed in the liver. Around the central vein, a distinct necrotic zone was formed. Hepatocytes were also degenerated mildly. Pyknosis and karyolysis was also observed to some level while cloudy swelling and edema were more pronounced. Around the portal tract was seen a focal collection of lymphocytes while mild infiltration of RBCs and inflammatory cells was observed in central vein. Mild degenerative changes with strong focal collection of lymphocytes and inflammatory cells were revealed in interstitium.

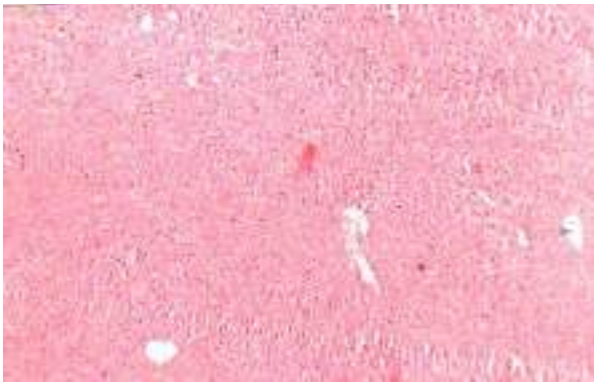

Fig: 5 T.S Passing through the liver infected with dose of 500 embryonated eggs showing dilation of central vein after 15 days.

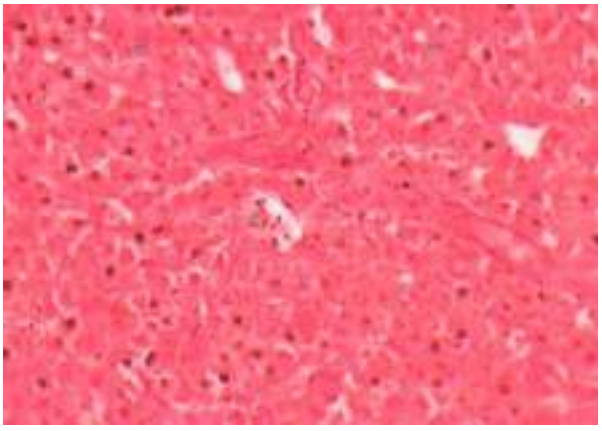

Fig: 6 T.S Passing through the liver infected with dose of 500 embryonated eggs showing infiltration of RBC's in central vein, pyknosis and karyolysis after 15 days.

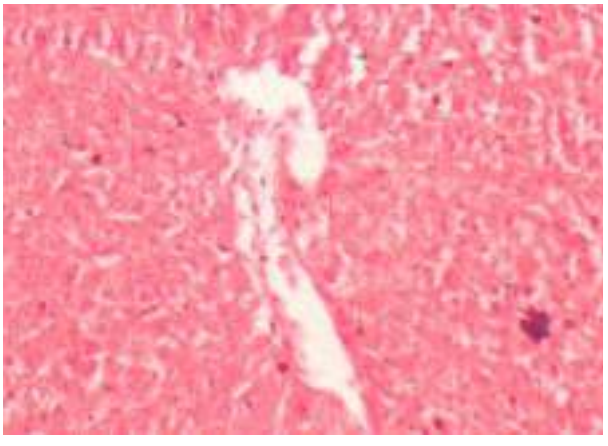

Fig: 7 T.S Passing through the liver infected with dose of 500 embryonated eggs showing pyknotic nuclei, edema and cloudy swelling after 15 days.

On 30th day after infection, the central vein was dilated and ruptured at some of the places. Infiltration of RBCs and focal collection of lymphocytes was observed. Degeneration was observed around the central vein of liver and also a fibrotic layer was formed around it. At some places hepatocytes have undergone mild degenerative changes with cloudy swelling. In some hepatocytes, necrosis was observed. Pyknosis, karyolysis and karyorrhexis were more pronounced. Focal collection of lymphocytes and inflammatory cells and other severe changes were observed around portal region of the liver. Around the inner lining of portal tract also, a thick band of fibrotic layer was formed. In the hepatocytes, atrophid nuclei and edema were more pronounced.

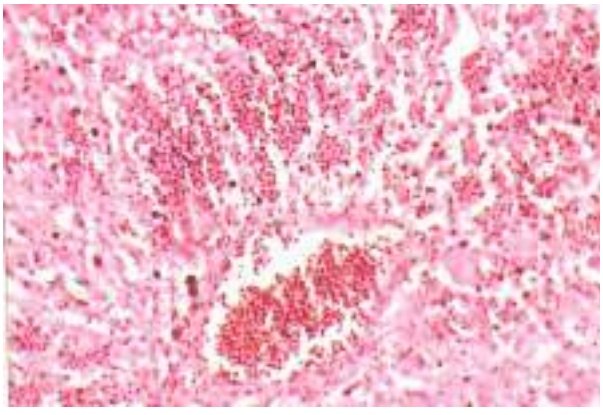

Fig: 8 T.S Passing through the liver infected with dose of 500 embryonated eggs showing fibrotic layer around the central vein, edema, infiltration of RBC's and focal collection of lymphocytes after 30 days.

On 45th day after infection, the pathological changes in liver of a catfish were severe. Edema with focal collection of lymphocytes was observed, Portaltract is lined by fibrotic layer. Dilation of central vein more pronounced. It is also surrounded by thick band of fibrotic layer and chronic inflammatory cells. Lumen of central vein is also filled with infiltrating RBCs and inflammatory cells. Mild degenerative changes were also observed around central vein. Necrosis in hepatocytes with pyknosis, karyolysis, karyorhexis was seen. Necrotic cells and inflammatory cells around the portal tract also marked some degenerative changes. Edema between sinusoidal cells was observed.

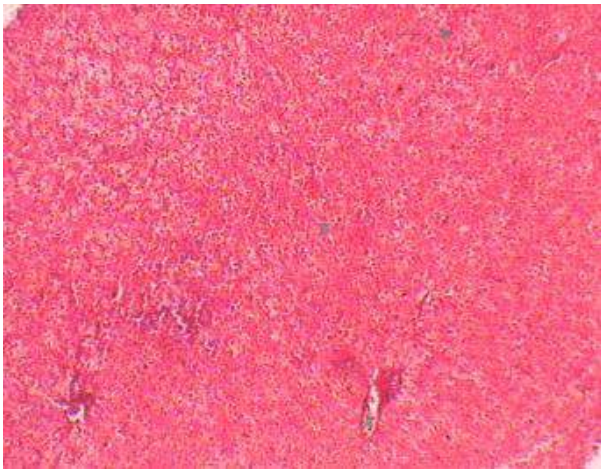

Fig: 9 T.S Passing through the liver infected with dose of 500 embryonated eggs showing edema, focal collection of lymphocytes and fibrotic layer around the portal tract around the central vein, edema, infiltration of RBC's and after 45days.

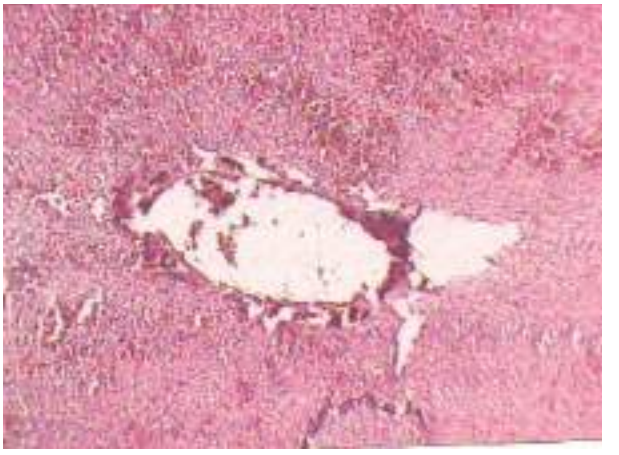

Fig: 11 T.S Passing through the liver infected with dose of 500 embryonated more dilation of central vein, edema, infiltration of some inflammatory cells in central vein and fibrotic layer around the central vein after 60 days.

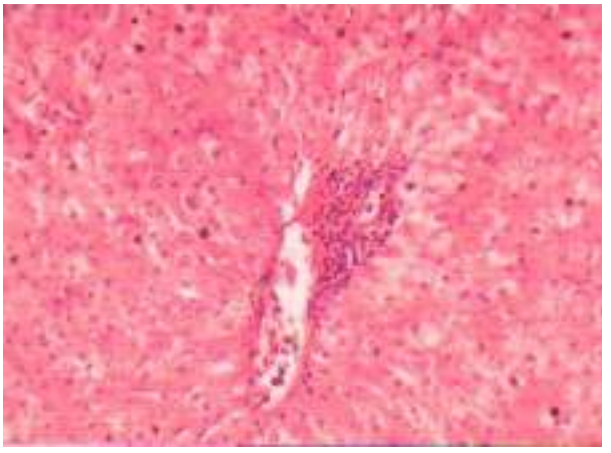

Fig: 10 T.S Passing through the Liver infected with dose of 500 embryonated eggs showing dilation of central vein, fibrotic layer around the central vein and atrophic layer after 45 days.

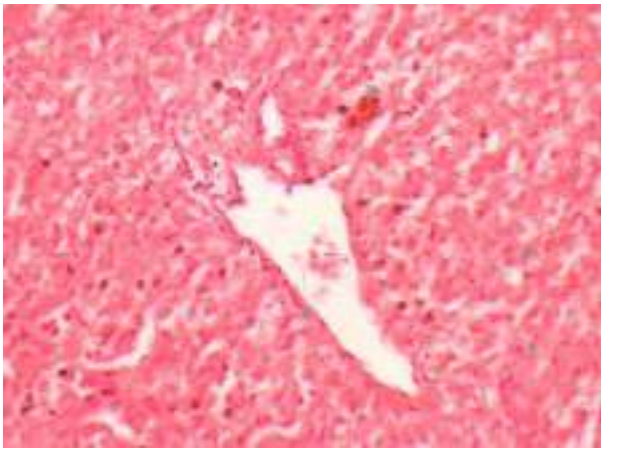

Fig: 12 T.S Passing through the liver infected with dose of 500 embryonated shrinkage of nuclei, fibrotic layer around the portal tract, focal collection of lymphocytes and pyknotic nuclei after 60 days.

After 60th day of infection, edema and severe degenerative changes around the central vein was observed. In the liver of infected *Clarias batrachus* will be seen infiltration of inflammatory cells in central vein, marked dilation and rapture of central vein at various places was also observed.

Fibrotic layer was clearly visible around the central vein. Pyknosis and karyolysis were seen. Focal collection of lymphocytes and edema were seen. As necrotic cells were observed around the portal tract and vein, degenerative changes were quite marked.

Kidney

Trunk kidney of the normal *Clarias batrachus* is composed mainly of haemopoeitic tissue, nephrons, convoluted tubules and collecting ducts. Renal capsule, a short neck and convoluted tubules forms a nephron. Glomerulus and Bowman's capsule together forms a renal capsule. Glomerulus is made up of central rounded compact mass of numerous mesangial cells which are surrounded by the tufts of glomerular capillaries. On the other hand, Bowman's capsule is composed of thin

squamous epithelium with outer peritoneal and inner visceral layers. The proximal convoluted tubule consisted of cuboidal epithelial cells with basal nuclei, luminal surface being lined by well-developed brush border. The distal convoluted tubule also consists of cuboidal epithelial cells which occupied only one third of the complete tubule. The cytoplasm is slightly granular in the epithelial ling cells. After 15 days of infection, the renal tissue of infected fish showed numerous glomeruli or variable sizes, cloudy swelling in tubules, edema, vacuolar/atrophic degeneration, enlargement of bowman's capsule, fibrosis, mild degenerative changes in PCT as well as DCT and necrotic changes in PCT, increased granulation and hyperplasia in PCT.

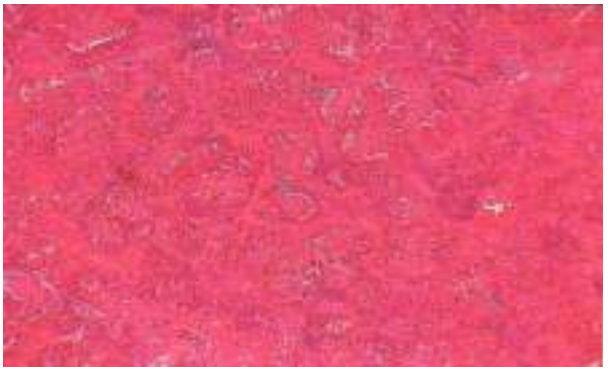

Fig: 13 T.S Passing through the Kidney infected with dose of 500 embryonated eggs showing variable size of glomeruli, cloudy swelling in tubules, edema, vacuolar degeneration or atrophic degeneration occurs throughout the section and fibrosis after 15days.

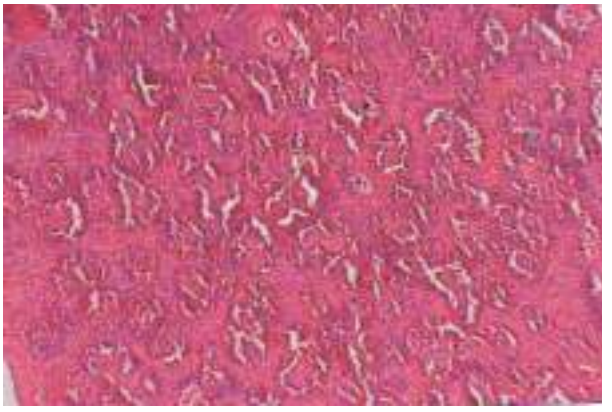

Fig: 14 T.S Passing through the Kidney infected with dose of 500 embryonated eggs showing mild degenerative changes in distal convoluted tubule, proximal convoluted tubule and enlarges size of Bowman's Capsule after 15days.

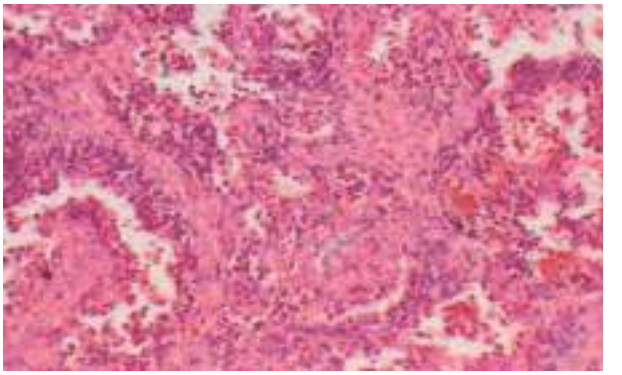

Fig: 15 T.S Passing through the Kidney infected with dose of 500 embryonated eggs showing mild degenerative changes in tubules necrotic changes in PCT, increased granulation and hyperplasia in PCT, edema and cloudy swelling after 15 days.

After 30 days, rupture of Bowman's capsule wall, proliferation & shrinkage in glomeruli, necrosis, pyknosis, karyolysis, karyorrhexis in PCT. Cloudy swelling, edema and inflammatory lymphocytes were also seen.

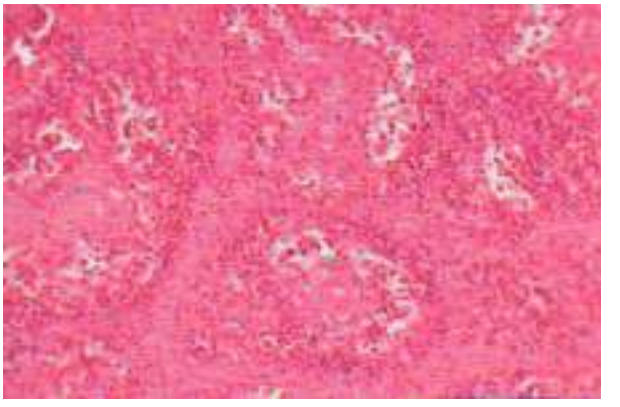

Fig: 16 T.S Passing through the Kidney infected with dose of 500 embryonated eggs showing necrosis, pyknosis, karyorrhexis, karyolysis in PCT, fibrosis, cloudy swelling and inflammatory lymphocytes after 30 days.

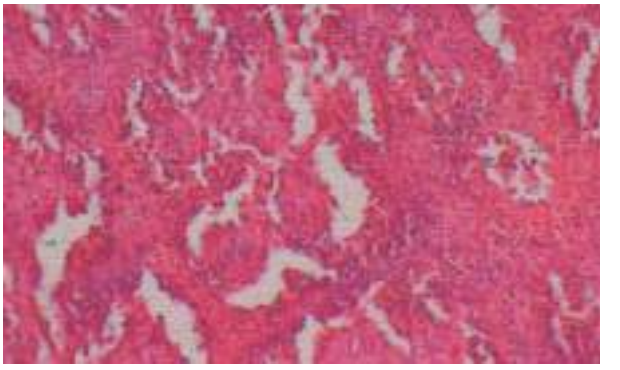

Fig: 17 T.S Passing through the Kidney infected with dose of 500 embryonated eggs showing proliferative and shrinkage in glomeruli, edema, necrosis, vacuolization in PCT and cloudy swelling after 30 days.

After 45 days, cloudy swelling in glomeruli and interstitium, infiltration in RBCs in intralobular vein, necrosis in PCT, vacuolisation in epithelial lining cells and necrotic haemopoietic tissue were observed as pathological changes.

Fig: 18 T.S Passing through the Kidney infected with dose of 500 embryonated eggs showing variable size of glomeruli, cloudy swelling in glomeruli, infiltration of RBC's in intralobular vein and necrosis in PCT after 45 days.

After 60 days, alterations were pronounced showing dilation in blood vessels, atrophy, shrinkage in pyknosis, karyorrhexis, karyolysis, vacuolisation, cloudy swelling, rupture of intralobular vein, necrosis in few PCT. Aggregation of lymphocytes and distinct inflammatory edema was also observed after 60 days of infection.

CONCLUSION

In the present study, congested blood vessels, glomeruli, edema and clody swelling were observed after 15th, 30th, 45th and 60th day of post infection with *Procammalanus* in *Clarias batrachus*. After 60 days these alterations and pathological changes were more pronounced also showed necrosis in few Proximal Convoluted Tubules (PCT), atrophy and shrinkage in glomeruli.

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EFFECT OF PHOSPHORUS AND PHOSPHORUS SOLUBULISING BACTERIA ON VEGETATIVE GROWTH OF FENUGREEK (TRIGONELLA FOENUM– GRAECUM L.) CV. LOCAL AGETI UNDER PRAYAGRAJ CONDITION

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ABSTRACT

An experiment was carried out in the Department of Horticulture. Kulbhaskar Ashram Post Graduate College, Prayagraj during 2018 in Rabi season. All the treatments and their combinations were significantly superior over control. Three parameters that is, Plant height, Number of branches per plant and Biomass/ green yield per hectare were assessed. Higher doses of Phosphorus were better in all three parameters . In P4 (55 kg P/ha) plant height was 24.49 cm and 27.50 cm at 60 and 90 days respectively. In B2 that is Spot placement of PSB the plant height was 19.43 cm and 22.93 cm at 60 and 90 days after sowing. Interaction effects of Phosphorus and PSB were far better than that of single application of both the factors. Usual practice of applying Biofertilizers as seed treatment was not better in comparison to Spot placement in hole before seed pacing in the same hole. P3x B2 interaction yielded best result in terms of plant height (40.94cm, 43.11cm) , number of branches/plant (9.62 , 11.03) and yield Q/ ha. (45.09 ,46.65) at 60 and 90 days after sowing. Second best result was recorded in P4x B2 interaction and showed best result in terms of plant height (36.71cm, 37.47 cm) , number of branches/plant (8.95, 9.10) and yield Q/ ha. (36.42, 38.95) at 60 and 90 days after sowing. Findings indicated that phosphorus Use Efficiency is significantly influenced by micro flora especially PSB . PSB not only mineralized the soil essential nutrients but also make yield sustainable.

Keywords : *PSB, fenugreek, effect.*

INTRODUCTION

Methi/fenugreek (*Trigonella foenum–graecum* L.) is a leguminous crop and has immense ability to fix atmospheric nitrogen in ideal condition of soil (moisture, PH, organic matter and other several factors).*Rhizobium melilotii*s a suitable strain to form root nodules in Methi plant. For efficient biological nitrogen fixation legume (methi)

needs more phosphorus as it is required for energy transformation in nodules of fenugreek plant. Besides, phosphorus also plays a significant role in root development, nutrient uptake and plant growth. Most of the soils have found deficient in phosphorus either due to inherent inability of soil are abnormal soil conditions as PH and other factors of soil. Even poor soil micro flora may adversely affect mineralization and availability of the phosphorus to the plant. The mineral sources are non renewable unlike nitrogen. So , there is need to enhance Phosphorus Use Efficiency e of applied fertilizer requires enhanced phosphorus acquisition from the soil by crops for proper growth and development . Among the nutrients, deficiency of phosphorus in soil has an adverse impact on legume production. Yadav et al. 2017. Phosphorus is essential for functioning of plant cells. Phosphorus is essential for carbohydrate metabolism (Vance et al. 2003). In suboptimal conditions reduced vegetative and reproductive growth of the plant (Vance et al. 2003). Phosphorus is directly associated with Biological Nitrogen Fixation of Methi crop .A large amount of phosphorus is required for metabolic pathways of energy transfer that takes place during nodules functioning**.** Phosphorus involves in photosynthesis regulation through energy transfer . Phosphorus has great influence on resistance to soil borne diseases and nucleic acid synthesis and plant maturity. Phosphorus increased the size and number of nodules **.** It acts as ingredients for Rhizobium bacteria to convert atmospheric nitrogen to ammonium **.** It also decreased time needed for active nodules development (Tang et. al. 2001). Proper phosphorus application found to increase yield 29.2 to 54.6% in pigeon pea and in Beans 62% .

Keeping above points in view, the experiment on Phosphorus application along with Phosphorus Solubilizing Bacteria was taken, so that the sole impact of both can be assessed at a time and draw certain conclusions about maximum methi biomass production with optimum utilization of resources.

MATERIALS AND METHODS

The experiment was conducted during 2018 in Rabi season at the Department of Horticulture Kulbhaskar Ashram Post Graduate College, Prayagraj. Soil was analyzed for nutrient application. 40 kg nitrogen and 45 kg Phosphorus per hectare was recommended on the basis of soil analysis. PH of soil was 7.8and the soil was loam, deep with medium organic matter content. No organic matter was applied during experiment. The experiment was laid out in factorial randomized block design with 3 replications. Seeds of Methi were conditioned by PSB. For this, seeds were soaked in plain water for 12 hours, after that soaked seeds were wrapped in PSB powder and kept in moist cotton cloth for 24 hours. In spot application**2**5ml 10% PSB solution was poured in hole in which one methi seed was already drilled. After that the Sowing is spot was firmly pressed to avoid aeration. Seeds were drilled at 3cm deep in 30 x 15 cm spacing. All the recommended phosphorus dozes were applied in row in 6 cm through khurpi and leveled properly. This was done just before drilling of seeds. Seeds were sown 25 October. Weeding, irrigation and hoeing were done timely and need based. Nitrogen in the form of Urea was divided into two equal dozes and first half dose was applied 10 days after sowing and second half dose 20 days after sowing through broadcasting .

RESULTS AND DISCUSSION

Phosphorus impacts significantly fenugreek plant. Vigour was far better with higher doses of phosphorus application. Interaction effects were remarkably better. All the treatments and their combinations were significantly superior over control. Three parameters that is, Plant height, Number of branches per plant and Biomass/ green

yield per hectare were assessed. Combination doses of Phosphorus were better in all three parameters. In P4 (55 kg P/ha) plant height was 24.49 cm and 27.50 cm at 60 and 90 days respectively. In B2 that is Spot placement of PSB, the plant height was 19.43 cm and 22.93 cm at 60 and 90 days after sowing. Interaction effects of Phosphorus and PSB were far better than that of single application of both the factors. Usual practice of applying Biofertilizers as seed treatment was not better in comparison to Spot placement in hole before seed pacing in the same hole. This might be due to better mineralization of fixed phosphorus already present in non available form in the soil. Similar results were also observed by Bhunia et al.2006; Girdhar and Sharda 2009 and Khiriya & Singh,2003.P3x B2 interaction yielded best result in terms of plant height (40.94cm, 43.11cm), number of branches/plant (9.62, 11.03) and yield Q/ ha. (45.09, 46.65) at 60 and 90 days after sowing. Optimum dose of Phosphorus fertilizer and better conditioning of seed might be the cause of the vigour. Findings were in conformity with the findings of Kumar et al.2009;Mehta et al. 2011 and Rathore & Manohar1998.Second best result was recorded in P4x B2 interaction and showed best result in terms of plant height (36.71cm, 37.47 cm), number of branches/plant (8.95, 9.10) and yield Q/ ha. (36.42, 38.95) at 60 and 90 days after sowing. Findings indicated that phosphorus Use Efficiency is significantly influenced by micro flora especially PSB. PSB not only mineralized the soil essential nutrients but also make yield sustainable. Results were in line with Mehta et al.2012; Rathore & Manohar1989; Tang et al. 2001; Vance et al. 2003 and Yadav et al.2017.

Table - 1 : Effect of Phosphorus and Phosphorus Solubulising Bacteria on vegetative growth of Fenugreek ((*Trigonella foenum– graecum* L.) cv. Local Ageti under Prayagraj conditions.

Treatments	Plant height (cm.)		Branches/plant (No.)		Fresh weight/Yield (Q/ha.)	
	60DAS	90DAS (FBD)	60DAS	90DAS (FBD)	60DAS	90DAS (FBD)
	Phosphorus					
P0	15.75	19.10	2.33	2.97	23.47	23.95
P1	18.23	20.12	3.12	3.88	24.56	25.33
P2	21.09	24.14	4.25	4.99	25.36	26.34
P3	23.19	26.13	4.97	5.12	26.92	27.56
P4	24.49	27.50	5.11	5.98	28.42	29.31
SEm+-	1.03	1.05	0.95	0.69	0.98	0.99
CDat5%	2.08	2.11	1.85	1.32	1.87	1.83
	Phosphorus Solubulising Bacteria (PSB)					
	60DAS	90DAS (FBD)	60DAS	90DAS (FBD)	60DAS	90DAS (FBD)
B0	16.31	19.34	2.91	3.85	23.59	24.01
B1	17.97	20.71	3.94	4.13	25.02	25.58
B2	19.43	22.93	4.78	5.11	26.23	27.12
SEM+-	1.02	1.06	0.93	0.94	1.05	1.01
CDat5%	2.11	2.20	1.23	1.23	2.13	1.89

Table - 2 : Interaction effect of Phosphorus and Phosphorus Solubulising Bacteria on vegetative growth of Fenugreek ((*Trigonella foenum– graecum* L.) cv . Local Ageti under Prayagraj condition.

Treatment	Plant height (cm.)		Branches (No.)		Fresh weight/Yield (kg.)	
	60DAS	90DAS (FBD)	60DAS	90DAS (FBD)	60DAS	90DAS (FBD)
	Phosphorus x PSB					
P1xB1	26.77	29.79	4.56	5.66	26.22	27.89
P1x B2	28.25	31.23	5.66	6.58	28.15	30.22
P2x B1	30.12	37.11	5.99	7.12	29.36	31.45
P2x B2	32.31	38.15	6.36	7.85	31.44	32.09
P3xB1	34.12	39.51	7.65	8.95	33.32	35.02
P3x B2	40.94	43.11	9.62	11.03	45.09	46.65
P4x B1	35.89	36.41	8.56	8.99	34.98	36.55
P4x B2	36.71	37.47	8.95	9.10	36.42	38.95
SEM+-	1.65	1.45	1.06	1.03	1.10	1.23
CDat5%	2.36	2.22	2.05	2.01	2.11	2.33

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EVALUATION OF DROUGHT STRESS ON GROWTH, PHOTOSYTESIS AND WATER RELATION IN TOMATO (SOLANUM LYCOPERSICUM L.) GENOTYPES

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ABSTRACT

According to the results it can be concluded that under stress conditions plant makes morphological and physiological changes to survive under stress. Tolerant species make remarkable changes for adaptation in stress condition. A ramified root system has been implicated in the drought tolerance species due to its ability to extract more water from soil and its transport to above ground parts for photosynthesis. In addition to other factors, changes in photosynthetic pigments are of paramount importance to drought tolerance. Of the two photosynthetic pigments classes, carotenoids show multifarious roles in drought tolerance including light harvesting and protection from oxidative damage caused by drought. Thus, increased contents specifically of carotenoids are important for stress tolerance. Similar observation was there in case of RWC, which decreases in tolerant species to maintain water balance in plants.

Keywords : *Tomato, physiological condition, evaluation*

INTRODUCTION

Stress is an altered physiological condition caused by factors that tend to disrupt the equilibrium. Strain is any physical and chemical change produced by a stress (Gaspar *et al*., 2002). Stress being a constraint or highly unpredictable fluctuations imposed on regular metabolic patterns cause injury, disease or aberrant physiology. Stress can be biotic or abiotic in nature. Abiotic stresses such as salinity, drought, chilling and oxidative adversely affect plant growth and development (Latif *et al*., 2016). A modest evaluation suggests that nearly 90% of global rural land area is affected by abiotic stress factors at some point throughout the growing period (Cramer *et al*., 2011). Abiotic stresses lead to specific genetic responses thereby resulting in an altered gene expression and their translation products in plants to help them adapt to the environment (Shah *et al*.,2011).Water shortage is predicted as the most severe environmental problem for the 21st century and drought is a major abiotic factor that limits crop production (Yuan *et*

al.,2010). Drought is an environmental stress which is a major barrier to productivity of agricultural crops throughout the world (Noaman *et al*., 2004).Tomato (*Solanum lycopersicon*), a member of the family Solanaceae is one of the most important vegetable crops grown and consumed all over the world (Kulkarni and Deshpande, 2007) and also a well-studied crop species in terms of genetics, genomics, breeding and molecular biology investigation. Although tomato can be successfully cultivated around the world, its growth and development is rather sensitive to a number of environmental stresses, especially drought, salinity, and extreme temperatures (Foolad, 2007). Water-deficit has a profound effect on tomato production worldwide (Sanjaya *et al*.,2005) and tomato plants fail to produce high yields in a fragile ecosystem (Foolad, 2007). Furthermore, most of the commercial tomato cultivars are moderately to highly sensitive to drought stress although differences between tomato cultivars have been reported. (Rus-Alvarez and Guerrier, 1994; Cano *et al*., 1996). In the present study we measured the early response of certain parameters associated with growth, photosynthesis, chlorophyll fluorescence and plant water relation in tolerant and susceptible tomato genotypes in relation to drought stress.

MATERIALS AND METHODS

Plant materials and stress conditions:-

The tomato genotypes H-86 (Kashi Vishesh) and EC-520061 seeds were obtained from ICAR-Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India, and used in present study. The plants were raised in a cultivation chamber under controlled conditions with relative humidity of 50 %, at 25°C/15°C (day/night), and a 16 h/8 h photoperiod with a photon flux density of 350 µmol m−2 s−1. Tomato plants were grown till attaining true leaf stage, transplanted one each in 40 pots, the diameter and height of the pots was 22 cm and 23.8 cm respectively. Under these condition progressive water-deficit stress treatments began after 50 d of germination when plants were at the late vegetative stage (before flowering), in triplicate, by withholding water for 7, 14, 21, or 28 days. The control treatment (well-watered:0d) was watered daily to receive approximately 80 % field capacity irrigation; whereas 7, 14, 21, or 28 days drought stress corresponded to about 40, 25, 15, or 10 % field capacity soil moisture, respectively as estimated by method of (Coombs *et al*.,1987). The plants received 1 liter water on release of the drought stress. Leaf samples were harvested from each treatment and instantly kept in liquid nitrogen and preserve at -80 °C in anticipation of their further analysis. The experiments were carried out in three different biological replicates.

Determination Root and Shoot Length

Root and shoot length of tomato plant which was determined by taking out plant at 10 leaf stage subjected to water withdrawal for 21 days from the soil with intact roots and were used for the measurement of root and shoot length

Determination of Dry and Fresh Weight Ratio

The plants taken for root and shoot length measurement were brought to the lab. The root, shoot and parts of the plants were separated carefully and their fresh weight was recorded. Respective plant parts were dried in an oven at 80°C temperature for 48 h and dry weight was recorded.

The ratio of dry and fresh weight was calculated according to the formula given by Kausar *et al*. (2012) with minor modification:

RDW/RFW % = (RDW/RFW) × 100

SDW/SFW % = (SDW/SFW) × 100

Where, RDW is root dry weight, RFW is root fresh weight, SDW is shoot dry weight, SFW is shoot fresh weight.

Determination of Electrolyte leakage (EL)

Electrolyte leakage was determined by

using a conductivity meter (CM-180, Elico, India) according to the operating instructions. Ten leaf discs of equal size from fully expanded fresh leaf were placed in 25 ml deionized water. The conductivity of the water was assessed after keeping for a 15-min vacuum filtration (VF), and thereafter autoclaving at 121°C for 30 min (AW). The EL was calculated by the equation: EL (%) = (VF/AW) ×100.

Determination of Photosynthetic Pigments *(Chlorophyll and Carotenoids)*

For chlorophyll and carotenoid estimation, leaf samples (300 mg) were crushed in 80% chilled acetone using a mortar and pestle. Absorbance of supernatant was read at 663, 645 480 nm and 510 nm calculated according to Lichtenthaler (2001). Chlorophyll and carotenoid contents (mg g-1 fresh weight) were calculated using the formula:

Chlorophyll A (mg g-1) = [(12.7 ×*A663) - (2.69 * A645)]

Chlorophyll B (mg g-1) = [(22.9 * A645) - (4.68 * A663)]

Total Chlorophyll = [(20.2 *A645) - (8.02 *A663)]

Carotenoid (mg g-1) = [(7.6*A480) - (1.49*A510)]

Where A663 stands for absorbance at 663 nm; A645 stands for absorbance at 645nm; A480 absorbance at 480nm; and A510 absorbance at 510 nm, respectively.

Determination of Chlorophyll fluorescence *(fv/fm)*

Photosynthetic efficiency was determined using a portable Handy Plant Efficiency Analyzer (Hansatech Instruments, King's Lynn, Norfolk, United Kingdom). The leaves were darkly adapted for 30 min using leaf clips at adaxial side. The red light were used to irradiates the leaf surface, the fluorescence signal generated was collected at excitation irradiance, set at 3000 µmol m−2 s−1 from the same surface. Minimum (*F0*) and maximum (*Fm*) chlorophyll fluorescence of dar adapted leaf were recorded and maximum quantum efficiency of photo system II calculated according to the formula $Fv/Fm = (Fm-F0)/Fm$ (Maxwell and Johnson 2000).

Determination of Relative Water Content (RWC)-

The RWC was measured following the method of Barrs and Weatherly (1962). Ten leaf discs from fully expanded fresh leaf, immediately after harvest, were weighed to determine the fresh mass (FM). The leaf discs were floated on double distilled water (50 ml) inside a closed Petri dish for 16 h under dim light (~10 µE m–2 S–2) at 25°C. At the end of the imbibition period, the leaf discs were blotted dry and weighed to obtain the turgid mass (TM). The leaf discs were dried in a forced-air oven at 80°C for 24 h, to obtain the dry mass (DM). The RWC was calculated by the equation: RWC (%) = [(FM–DM)/(TM–DM)] ×100

RESULTS AND DISCUSSION

Effect of drought on plant morphology-

Tomato genotypes were exposed to drought stress by withholding the water for different days of interval such as 7 days, 14 days, and 21 days. Both the genotypes have different capacity to bear the drought stress. The drought tolerant plants (EC-520061) could clearly withstand the 7 days drought treatment very well without showing any wilting symptom, while the susceptible plants wilted. Wilting symptoms in the tolerant plants were delayed; plants remained greener, and continued to grow after the 14 days of drought stress, while the susceptible plants (H-86) began to show symptoms of greater tissue injury, as determined by leaf yellowing Even after 21 days of severe drought stress, the upper 4–5 leaves of the tolerant plants remained green, whereas, in susceptible plants, all the leaves except upper two became yellow. After 28 days of continuous drought stress treatment, all pots were watered simultaneously to investigate plant

recovery. The tolerant plants (EC-520061) recovery was evident by new branches and green leaves, while the susceptible plants having exposure of 21 days could not recover and died whereas plants exposed for 7 and 14 days recovery was slow.

Effect of drought on Root and Shoot length-

The result shows that on exposure to drought root length of EC-520061 genotype of tomato goes on increasing as the day of water withholding increases compared to H-86. During control condition i.e. at 0 days root length was 56 cm which increased further to 61cm, 73cm and 81 cm for 7, 14 and 21 days of drought exposure respectively, whereas in case of H-86 there is no such significant increase in root length was observed during drought stress condition compared to control i.e. at 0 days root length 57cm at on 21 days of drought exposure observed root length was 41cm as shown in Table (1). The result shows that drought tolerant lines have longer root length then susceptible genotypes. These root elongation helps the drought tolerant to extract water from deep soil. Similar results were reported on wheat cultivars (Almaghrabi, 2012) pearl millets (Lelia, 2007) and chickpea (Macar *et al.,* 2009). Production of a prolific root system under drought stress increases water uptake from soil, maintain requisite osmotic pressure, maintain plant biomass development, and accelerate the plant growth during the early growth stages (Jaleel *et al*., 2009).

In case of shoot length no such significant difference was observed on exposure to drought till 14 days compared to control plant i.e. (0 days) in almost both the genotypes of tomato. In H-86 plant shoot length was almost same from 0 days to 7 and 14 days of exposure but it decreases on 21 days of drought exposure i.e.75 cm, 77cm, 78cm and 62cm respectively as shown in Table (2). In case of EC-520061 the shoot length almost remain same for 14 days of drought exposure but slight decrease in shoot length was observed from control i.e. (0 days) to 21 days of exposure. The results shows that drought have negative effect on shoot length of tomato genotype. Similar results were observed in case of tomato genotypes by (Kulkarni and Deshpande, 2007). A comparatively well established above-ground part of a plant is the integrative effect of root adaptability to exploit the available water (Ekanayake *et al*.,1985). In the present experiment increase in root length while the decrease shoot length in parallel was observed by (Matsui and Singh 2003) also observed similar trend of root distribution in various cowpea genotypes grown under drought condition. They also observed that root distribution shifted downward under water during water withdrawal condition. Deep and prolific root system was found to be associated with enhanced avoidance of terminal drought in chickpea. The decrease in shoot length under drought condition may be due to the suppression of cell expansion and cell growth that could be due to plants response to low turgor pressure (Jaleel *et al*., 2008).

Effect of drought on Dry and Fresh weight ratio-

In present study of shoot and root dry weight ratio the ratio goes on increasing as observed that highest root ratio was in EC-520061 at 21 days of drought exposure i.e.13.04% and in H-86 it was 9.38%. Similar observation was there in case of shoot ratio in H-86 and EC-520061 at 21 days of exposure was 19.49% and 23.49% respectively whereas in control condition (0 day) in EC-520061 and H-86 was 20.34% and 15.06% respectively The above results shows that plants exhibiting better tolerance against drought showed superior water withholding capacity. Any plant part retaining more water will exhibit high ratio of dry and fresh weight compared to those retaining less water. In present experiment it was observed that dry and fresh weight ratio of root, and shoot increases as days of water

withdrawal increases, with maximum increase in tolerant genotype compared to susceptible. According to (Kravic *et al*., 2013) relative increase in dry and fresh weight ratio of root and shoot due to water withdrawal could be considered as an indicator of the osmotic stress level.

Table - 1 : Effect of elevated water-deficit on root length and ratio of root dry and fresh weight of tomato genotypes. The results represent mean ± standard error of triplicate measurements.

Genotype	Root Length (cm)				Genotype	Root Dry and Weight Ratio (%)			
	0	7	14	21		0	7	14	21
H-86	57±3.24	55±3.28	48±3.96	41±2.33	H-86	6.25±1.49	8.06±2.83	8.78±1.19	9.38±2.53
EC 520061	56±2.39	61±2.71	73±1.17	86±2.14	EC 520061	8.4±1.17	10.0±1.09	10.34±2.16	13.04±2.51

Table - 2 : Effect of elevated water-deficit on shoot length and ratio of shoot dry and fresh weight of tomato genotypes. The results represent mean ± standard error of triplicate measurements.

Genotype	Shoot Length (cm)				Genotype	Root Dry and Weight Ratio (%)			
	0	7	14	21		0	7	14	21
H-86	75±4.11	77±4.62	78±4.02	62±3.96	H-86	15.06±2.01	15.46±1.89	16.10±1.61	19.49±2.01
EC 520061	107±4.02	106±3.89	101±3.78	93±4.54	EC 520061	20.34±2.84	21.14±1.08	23.44±2.77	23.49±2.28

Effect of drought on Electrolyte Leakage-

Plant cell maintain electrolytes within cell membranes that are vital for proper functioning of cell. When the cells are subjected to stress, electrolyte leaks in the surrounding tissues, this leads to potentially irreversible condition and the loss of compartmentation and cell death (Noodén *et al*., 2004). Cells integrity and stability is quantified by measuring relative conductivity of leaked ions in water (Ristic and Ashworth 1993; Bajji *et al*., 2002; Rolny *et al*., 2011). Electrolyte leakage has been categorized as a valuable parameter for identification of stress tolerant cultivars in several crop species (Leopold *et al*. 1981; Stevanovic *et al*., 1997). EL measurement can also be correlated to various physiological and biochemical parameters in plant responses to various environmental condition such as anti oxidative enzyme synthesis (Liu and Huang 2000; Sreenivasulu *et al*., 2000).

In the present experiment lower extent of electrolyte leakage was found in EC-520061 then H-86 drought susceptible genotype of tomato under all three drought treatment. On exposure to drought H-86 plants shows higher increase in EL percentage as shown in fig 1. During well watered condition H-86 and EC-520061 has no significant difference in EL value. When exposed to drought, there is increase in EL % in both H-86 genotypes and EC-520061 to about 28.18% and 15.91% respectively, at 7 days of treatment in which remain constant to 14 days of exposure (i.e. 27.22%, and 17.41 %), but sudden increase in EL value is observed at 21 days of drought treatment the percentage goes to 82.94%. in H-86 genotype, and 52.20 in EC-520061 which is comparatively lower then H-86 genotype. The results of lower EL in EC-520061 events reflect

higher membrane integrity of these plants under the different drought stress treatments (Khare *et al*., 2010) compared to the corresponding drought susceptible plants as shown in fig 3. Valentovic *et al*., (2006) reported that the electrolyte leakage of the sensitive maize cultivar increased from 11 to 54%, but the increase in ion leakage of tolerant cultivar was not so high. Sreenivasulu *et al*., (2000) observed positive correlations between salt sensitivity and membrane damage in foxtail millet (*Setaria italica*) seedlings. (Quan *et al*., 2004) also found higher electrolyte leakage in drought stressed maize (*Zea mays* L.) plants than in plants grown under well watered conditions. Similar results were also observed in case of Borujerd *Kochia* ecotype , the data indicate a water stress-induced membrane injury, in Borujerd *Kochia* ecotype.

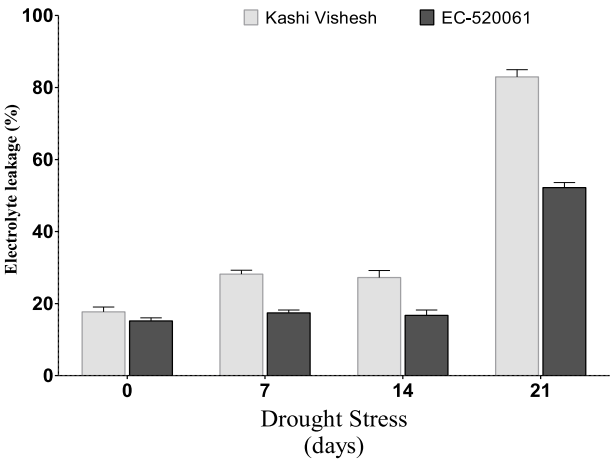

Fig.-1: Effect of water stress on Electrolyte Leakage activity in tomato genotype. The data are mean of three replicates +SE. And bars indicate Standard Error

4. Effect of drought on Photosynthetic Pigment Chlorophyll

Water deficit is a limiting factor, affecting morphological and physiological process in plants associated with plant growth and development, photosynthesis in particular (Toker and Cagirgan, 1998).Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers (Farooq *et al*., 2009). In relation to this foliar chlorophyll content of a plant plays a key role in affecting the performance of plant photosynthesis (Taiz & Zeiger 2006).

Photosynthetic pigments allow plants to absorb energy from light, so foliar chlorophyll content is a key factor affecting the performance of plant photosynthesis (Taiz & Zeiger, 2006).

In the present experiment Chlorophyll content A, B and total Chlorophyll values have been observed in both the genotypes. It has been observed that Chlorophyll A content have decreased in both the genotypes during water withdrawal condition; the percentage goes down from 69.1% to 50.17% from 7 to 14 days of stress condition and it further goes down to 33.5% at 21 days of water withdrawal condition in H-86 genotype, whereas in genotype EC-520061 the value is comparatively higher during stress condition, at 7 days of stress condition the Chl A is 79.18% and 73.15% at 14 days of stress condition but after 21 days of stress condition the % decreases to 65.30%. The results show that Chlorophyll A decreases in stress condition as shown in fig-2.

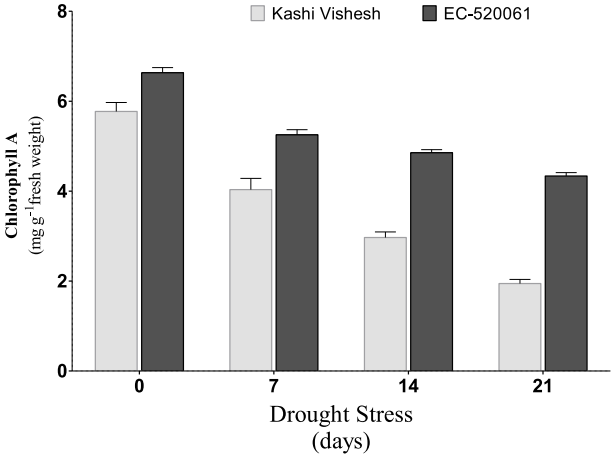

Fig. - 2 : Chlorophyll A activity in both the genotypes. The data are mean of three replicates +SE and bars indicate Standard Error.

In case of Chlorophyll B, the Chlorophyll percentage during stress condition (7-21days) in H-86 genotypes reduces from 74.5% to 68.4% , whereas in case of EC-520061 at 7 days of water withdrawal the percentage remain 94% at 14 days it goes to 80.3% but after 21 days of stress the value goes down to 67.1%. It can be observe that in both the genotypes chlorophyll percentage reduces but in case of EC-520061 after 21 days of stress the chlorophyll percentage is still higher than H-86 genotype as seen in Fig-3. Similarly in case of total chlorophyll at 7 days of stress condition in H-86 the chlorophyll percentage is 70.4%, whereas in EC-520061 the value is 90.7%, after 14 and 21 days of stress the percentage is 70.6 and 76.5% respectively in H-86, whereas in EC-520061 the chlorophyll percentage is 80.4% at 14 days of stress condition, further it reduces to 67.1% after 21 days of water withdrawal condition.

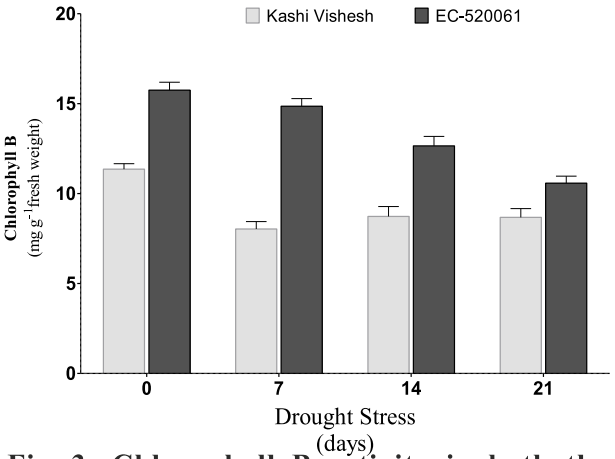

Fig.-3: Chlorophyll B activity in both the genotypes. The data are mean of three replicates +SE and bars indicate Standard Error.

The obtained results show that Chlorophyll A, B and total Chlorophyll value decreases during stress condition. Drought stress can alter the tissue concentrations of chlorophylls and carotenoids (Hussein *et al*., 2008). Drought stress inhibits Chl a/b synthesis and decreases the content of Chl *a/b* binding proteins, leading to reduction of the light-harvesting pigment protein associated with photosystem II (Sayed, 2003). Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger *et al*., 2002).Various reports have explained that drought stress significantly decreases the chlorophyll a, chlorophyll b and total chlorophyll content of different crops (Mafakheri *et al*., 2010) such as cotton (Massacci, 2008) and *Catharanthus roseus* (Jaleel *et al*., 2008a).Chlorophylls decreased significantly under higher water deficit in sunflower plants (Kiani *et al*., 2008). It is known that environmental stresses in terms of chlorophyll degradation have similar effects on plants. Zaeifizade and Goliov (2009) reported that resistant cultivars have more chlorophyll similar results have been obtained in case of EC-520061.Decreased or unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought (Kpyoarissis *et al*., 1995).

Effect of drought on Carotenoid Pigments-

The chlorophyll and carotenoid pigments are involved in harvesting light energy in plants (Tzvetkova-Chevolleau *et al*., 2007) and their content is related to plant drought tolerance (Saglam *et al*., 2011). The carotenoid play fundamental roles and help plants to resist drought stress (Jaleel *et al*., 2009). During stress condition in tomato, EC-520061 genotypes have higher carotenoid content than the H-86 genotype in all drought treatments as well as well watered plants, in EC-520061 the carotenoid percentage range 92.8-79.5% ,after 21 days the value goes to 74.5% , while in H-86 the carotenoid value is 71.2-66.0% in 7 to 21 days of water withdrawal condition.(fig-4) Water deficit stress reduces the tissue concentrations of chlorophylls and carotenoids (Havaux 1998; Kiani *et al.*, 2008), primarily due to the production of ROS

in the thylakoids (Reddy *et al.*, 2004).

β–carotene, a carotenoid, present in the chloroplasts of all green plants and is exclusively bound to the core complexes of PSI and PSII (Havaux, 1998). Protection against damaging effects of ROS at this site is essential for chloroplast functioning. β–carotene, in addition to function as an accessory pigment, acts as an effective antioxidant in PS and plays a unique role in protecting photochemical processes by sustaining them (Havaux 1998). A major protective role of β–carotene in photosynthetic tissue may be through direct quenching of triplet chlorophyll, which prevents the generation of singlet oxygen and protects from oxidative damage (Farooq *et al.*, 2009). Thus, higher carotenoids content are important for water-deficit stress tolerance. Exogenous application of water-deficit/pigment inducers brassinolide, uniconazole and methyl jasmonate improved the drought tolerance with increased activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase APX), ABA and total improved carotenoid contents in maize (Li *et al.,* 1998). Stronger decrease in total Chl and Car. contents have been reported in drought exposed sensitive common bean genotypes than the drought tolerant genotypes (Saglam *et al*., 2011).

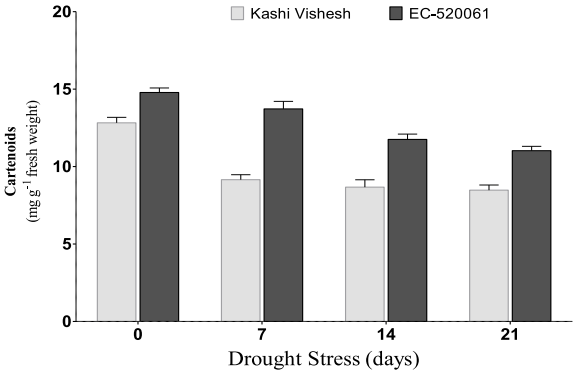

Fig.-4 : Effect of water stress on Carotenoid pigment activity in both the genotypes. The data are mean of three replicates +SE and bars indicate Standard Error.

Effect of drought on *Fv/Fm* Ratio (Chlorophyll Fluorescence)

Chlorophyll fluorescence is an image providing tool in plant phenotyping which image physiological phenomena interfered with photosynthetic apparatus and its associated metabolism (Cen *et al*., 2017). Chlorophyll fluorescence measurement is a non-destructive non-time consuming and relatively simple technique for studying the equilibrium between metabolic and energy evolving processes, that maybe affected by both temperature and drought stresses (Araus and Hogan, 1994; Flagella *et al*., 1995). (Martinez-Ferri *et al*., 2016) identified the disease severity in avocado leaves by using conventional chlorophyll fluorescence parameters and observe that *Fv'/Fm'* and *Fs/Fo* of avocado leaves decreased with the infection of white root rot.

Earlier studies have successfully applied multicolor fluorescence for evaluating fruit qualities (Lichtenthaler *et al*., 2012) pathogen attack (Ortiz-Bustos *et al*., 2017; Perez-Bueno *et al*., 2016) and nutrient and water deficiencies (Hsiao *et al*., 2010; Tremblay *et al*., 2012).

In the present experiment it has been observed that *Fv/Fm* ratio goes on decreasing as exposure to drought increases. It was observed that in EC-520061 *Fv/Fm* ratio was minimum on 21 days of water withdrawal (0.62) but it was higher than H-86 at same days of water interval (0.41). *Fv/Fm* ratio was almost same at 7 days of drought interval in both the plants but as days of drought exposure increases *Fv/Fm* ratio goes on decreasing, and the ratio was minimum in case of drought susceptible genotype i.e. H-86 at 21 days of water withdrawal condition as shown in table (3). The observed results are in coordination with (Liu *et al*.., 2012) who also observed a decline in *Fv/Fm* ratio in drought stressed plants of two maize cultivars.In the current study, with an increase in the degree of drought

stress, *F*V/*F*M, *Φ*PSII, and *q*P gradually decreased, suggesting that drought initiated the closure of the PSII reaction center, limiting electron transfer and reducing the light energy available for actual photochemical reactions in the PSII reaction center (Stefanov and Terashima, 2008). Maximum *F*V/*F*M is reflective of potential maximum photosynthetic capacity of plant leaves under non stressful conditions after a sufficient dark adaptation. *F*V/*F*M reflects the potential maximum photosynthetic capacity of plant leaves after a sufficiently dark adaptation (Sharma *et al*., 2015). *F*V/*F*M is relatively constant, generally between 0.75 and 0.78; but it also varies with plant cultivars. When plants are stressed, *F*V/*F*M decreases, depending on the plant cultivar and growth status. In this experiment, with the increase of drought stress, *F*V/*F*M decreased.

Table - 3 : Effect of elevated water-deficit on Fv/Fm ratio on genotypes of tomato. The result represents mean ± standard error of triplicate measurements

Genotype	*Fv/ Fm* Ratio			
	0	7	14	21
H-86	0.75±1.32	0.78±1.12	0.47±1.16	0.41±1.23
EC520061	0.79±1.93	0.77±1.71	0.68±1.71	0.62±1.24

Effect of drought on Relative Water Content-

RWC is the best criteria and most reliable indicator to access the water status in plants (Barrs and Weatherley 1962; Rampino *et al*., 2006). RWC can directly indicate the balance between water absorbed by plants and rate of transpiration (Arjenaki *et al.,* 2012). Sanchez-Rodriguez *et al*., (2010) reported that RWC was one of the best indicators in tomato plant for separating tolerant and sensitive cultivars. In case of drought lower RWC is the major factor in reduction of growth of stressed plants (Alexieva *et al*., 2001**).** The magnitude of RWC in drought tolerant plant is relatively high then drought susceptible plant.

The RWC of the drought tolerant line EC-520061 in well watered condition is 69%- 66% which is comparatively higher in comparison to other stress days , RWC value remains constant during the first 7 and 14 days of drought, (69%-66% and 68%-61% respectively) and then decreased at 21 days of drought progressively to about (55%-48%).The RWC value remain constant in drought susceptible line H-86 during well watered condition 0days (54.6%) and 7 days of stress conditions (53.3%) respectively, after 14 days of stress the value decreased to (46.54%) and finally at 21 days RWC value get reduced to(44.25%). The above results show that EC-520061 is more drought resistant and is highly adaptable to drought condition then H-86 genotype of tomato.

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EFFECT OF BIO-FERTILIZERS ON GROWTH, YIELD AND QUALITY OF SPINACH (BETA VULGARIS L.) CV. PUSAHARIT

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ABSTRACT

An experiment was conducted to know the effect of Bio-fertilizers on growth yield quality of spinach cv. PusaHarit. Different Levels of Bio-fertilizers &viz. Azotobactor and PSB were given as soil application and seed treatment. The data regarding effect of bio-fertilizers on growth and yield of spinach has been presented in table 1. It is obvious from table that height of plant (21.55, 27.66cm) number of leaves (10.88, 20.33) have been recorded maximum under T_7 (Azotobacter + PSB @ 2.5 lit/ha) in both stages at 30 and 40 DAS. It has been observed that maximum fresh weight of whole plant/(100.11g) yield per plot (3.85kg) and yield per hectare (64.34Q) was also found under T_7 (Azotobacter + PSB@2.5 lit/ha). The growth and yield parameters of spinach noted minimum under control (To). The data presented in table 2 showed that treatment T7 (Azotobactor + PSB@2.5 lit/ha) exhibit superiority over other treatments regarding maximum content of vitamin C (0.67IU), iron (24.62 mg/g), Chlorophyll (39.04 mg/cm2) and shelf life (5.20 days), while maximum Vitamin A content (735IU) was bagged in T8 (Azotobacter @ 12kg and PSB@ 2.5 lit/ha). All quality Parameters were noted minimum under Control (To).

Key words **:** *Spinach, biofertilizer, effect.*

INTRODUCTION

The spinach (*Beta vulgaries L.*) Commonly called as palak belonging to family Chenopodiaceae is one of the most popular vegetable Crop grown in India and other parts of the world as leafy vegetable. It is used fresh, canned or as frozen products. It is low in calories and with a high biological value extremely rich in antioxidants especially when Fresh steam quickly boiled spinach is a rich source of vitamin A. Iron and calcium and also contains appreciable quantity of ascorbic acid, riboflavin and small quantity of thiamin. The Leaves are bright green in colour lustrous fleshy and accepted by the varied groups of populations. The Crops can be harvested 6 to 7 times with application of Nitrogen after each harvest. (Thompson and Kelly 1957) Palak is valued among all leafy vegetables due to better returns, shortest life span, medicinal and nutritive value. The growth, yield and quality of palak leaf in a particular area depend upon the

genetic constitution of cultivar. environmental factors and adaptation of improved agro techniques or the management practices.

n recent years as the consumers are become more aware about the use of chemical free vegetables. Hence it become the need to sustain the production with minimum or no use of chemicals. The scaling cost of chemical fertilizers has compelled the formers to shift towards alternatives to chemicals forming. Bio-fertilizers are less expensive, eco friendly and sustainable compelled increasing yield, improving quality also help in improving the nutrient status of soil.

Azotobacter is an aerobic, free living gram negative bacterium which fixes nitrogen from atmosphere. Application of phosphate solubilizing bacteria (PSB) can help in reducing the input of chemical fertilizers as well as in maintaining better soil health. PSB increase the availability of phosphorus in the soil through secretion of phosphates enzyme. There are very limited studies available on use of Azotobecter and PSB in vegetable crops. The formers are in need of the information regarding type of formulation of bio-fertilizers should be used and effective method of application. In the light of above facts, lack of information on spinach and considering the importance of spinach for human health, it was felt necessary of generate the research based information regarding use of bio-fertilizers. Therefore the present investigation entitled" Effect of bio-fertilizers on growth, yield and quality of spinach *(Beta vulgaris L.*) cvPusaHarit was carried out.

MATERIALS AND METHODS

A field experiment entitled "effect of Bio-fertilizers on growth yield and quality of spinach *(Beta vulgaris L)*cv.PusaHarit" was conducted at Horticulture Farm, Kulbhaskar Ashram Post Graduate College, Prayagraj during rabi season 2020-21. The experiment was laid out in randomized block design (RBD) with 3 replications. Experiment comprises 10 treatments of different doses of Azotobacter and PSB. A recommended dose of fertilizers (RDF) 85:40:40 kg N:P:K was applied as control.

The solid form of Azotobacter and PSB was applied in soil as per treatment. Seed treatment of Azotobacter and PSB, to spinach seed was done before sowing. The liquid bio-fertilizers were mixed with water and applied to the plot as per treatments immediately after sowing of seeds.

Seeds were sown by opening furrows of 3 cm depth at spacing of 10 x10 cm by hand drilling and covered with fine soil. All cultural operations were performed time to time.

Data for recording of growth and yield attributes three plants were selected under each treatments randomly. The growth parameter viz. height of plant and number of leaves were noted at 30 and 40 days stages. The yield attributes i.e. fresh weight of whole plant, yield per plot and yield per hectare were recoded with the help of electronic balance and calculated accordingly. Quality characters viz. Vitamin A, Vitamin C, iron and chlorophyll content were estimated by various scientific methods. The observations were analyzed statistically.

RESULTS AND DISCUSSION

The data regarding effect of bio-fertilizers on growth and yield parameters of spinach have been presented in table 1. It is clear form table that maximum height of plant (21.55, 27.66 cm) was recorded under treatment T_7 (Azotobacter + PSB@2.5 lit/ha) at 30 and 40 DAS. which was significantly higher over other treatment and found statistically at per with treatments T_8 (18.00, 23.00) cm and T_3 (17.33, 22.44). The minimum plant height (12.96, 17.55cm) was noted under control (To) at both stages, 30 and 40 DAS. This finding is in

conformity with finding of Sharma et al (2011) and Negiet al (2018). Bio-fertilizers also influenced the number of leaves per plant. Highest number of leaves (10.88, 20.33) were also noted under treatment T_7 which was statistically at per with the treatments T_8 (9.22) and T_2 (9.22) to 30 DAS stage. The reduced number (minimum) of leaves per plant observed in RDF (control). The fresh weight of whole plant observed in RDF (control). The fresh weight of whole plant (100.11g), yield per plot (3.85kg) yield per hectare (64.34) and increased percentage were noted maximum under Treatment T_7 (Azotobacter + PSB@2.5 lit/ha), while minimum observations of these parameters were noted under control To. Similar results were also observed by Kumaret al (2017).

The quality attributes were also significantly affected by different levels of bio-fertilizers. The observations regarding quality parameters have been presented in table 2. It is obvious from table that treatment T_7 (Azotobacter +PSB@2.5 lit/ha) exhibited superiority over other treatments by gaining maximum content of moisture (80.85%), Vitamin C (0.67 IU/100g), iron (24.62 mg/g). Chlorophyll A (39.04 mg/cm^2) and shelf life (5.20 Days) while maximum vitamin A (735.00 IU/100g) was noted under T_8 (Azotobacter@12kg@ha and PSB@2.5 lit/ha). Table also showed that the inferior results were noted under control (To).

The growth and yield attributes of spinach influenced significantly by different levels of Azotobacter and PSB (Table 1). The highest growth of spinach 21.555, 27.66 cm was observed with the application of Azotobacter PSB @ 2.5 lit/ha. Similarly maximum yield (64.34 Q/ha) of spinach was noted when Azotobacter and PSB were applied jointly in liquid form @ 2.5 lit/ha. The solid form of Azotobacter and PSB @ 12 kg/ha was also influenced the growth and yield of spinach when applied as basal before sowing. The responses of bio-fertilizers on quality attributes of spinach is also clear in table 2. The quality parameters viz. Moisture content, Vitamin A and C content, Iron and

Table - 1 : Influence of Bio-fertilizers on Growth and Yield Parameters of Spinach.

Treat ments	Treatment Details	Height of plant (cm)		No. of leaves per plant		Fresh weight of whole plant (g)	Yield per plot (kg)	Yield per hectare (Q)	% increase over control
		30 DAS	40 DAS	30 DAS	40 DAS				
To	RDF (Control)	12.96	17.55	7.33	9.66	72.00	2.22	37.22	-
T_1	Azotobacter@ 12kg/ha	16.33	20.66	8.22	11.89	90.22	2.95	49.34	32.56
T_2	PSB@ 12kg/ha	15.67	19.22	9.22	11.77	99.11	3.16	52.78	41.81
T_3	Azotobacter + PSB@ 12kg/ha	17.33	22.44	7.78	17.55	91.77	3.55	59.33	59.40
T_4	Azotobacter + PSB as seed treatment	15.78	21.89	88.89	11.22	77.33	3.00	50.17	34.89
T_5	Azotobacter @ 2.5 lit/ha	14.89	18.33	8.44	11.00	85.22	3.00	50.17	34.89
T_6	PSB @ 2.5 lit/ha	14.11	18.11	7.44	9.89	93.44	2.98	49.78	35.75
T_7	Azotobacter + PSB@ 2.5 lit/ha	21.55	27.66	10.88	20.33	100.11	3.85	64.34	72.86
T_8	Azotobacter@12kg/ha and PSB@ 2.5 lit/ha	18.00	23.00	9.22	11.44	97.44	3.55	59.33	59.40
T_9	Azotobacter@ 2.5 lit/ha and PSB @12 kg/ha.	16.88	21.33	7.66	11.22	95.89	3.17	53.00	42.40
S.E.+		0.82	05.62	0.37	0.51	0.97	0.17	3.29	3.59
C.D. at 5%		2.43	1.86	1.09	1.52	2.88	0.50	9.77	9.89

Table - 2 : Influence of Bio-fertilizers on Quality Parameterss of Spinach.

Treatments	Treatment Details	Moisture Content (%)	Vitamin A (IU/100g)	Vitamin C (IU/100g)	Iron Content (mg/g)	Chlorophyll A Content (mg/cm^2)	Self life (Days)
To	RDF (Control)	56.92	699.00	0.44	10.10	34.47	2.20
T_1	Azotobacter@ 12kg/ha	65.90	703.00	0.49	10.15	34.34	3.87
T_2	PSB@ 12kg/ha	73.90	716.00	0.57	19.72	34.04	2.87
T_3	Azotobacter + PSB@ 12kg/ha	71.11	716.00	0.57	20.09	31.03	4.87
T_4	Azotobacter + PSB as seed treatment	62.12	709.00	0.53	22.49	36.47	3.53
T_5	Azotobacter @ 2.5 lit/ha	74.69	707.00	0.48	18.25	34.81	4.53
T_6	PSB @ 2.5 lit/ha	70.20	690.00	0.61	18.73	34.91	5.20
T_7	Azotobacter + PSB@ 2.5 lit/ha	80.85	707.00	067	24.62	39.04	5.20
T_8	Azotobacter@12kg/ha and PSB@ 2.5 lit/ha	61.72	735.00	0.53	18.13	36.27	2.87
T_9	Azotobacter@ 2.5 lit/ha and PSB @12 kg/ha.	73.52	705.00	0.56	21.23	38.18	2.73
S.E.+		0.60	4.39	0.03	1.09	0.56	0.39
C.D. at 5%		1.73	13.02	0.09	3.23	1.64	1.16

chlorophyll content greatly influenced by different levels and form of application of Azotobacter and PSB. Better quality of spinach was noted under treatment T_7 (Azotobacter + PSB@2.5 lit/ha) and T_8 (Azotobacter@12kg and PSB@2.5 lit/ha)

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A COMPARATIVE STUDY OF CHEMICAL QUALITIES OF BUFFALO AND SHEEP MILK

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ABSTRACT

A comparative study of chemical qualities of raw milk of buffalo and Sheep as conducted at Livestock production and management (unit), Department of NRM, faculty of agriculture, MGCGV Chitrakoot–Satna (M.P.) during January to February 2021. The objective was to find out the comparative chemical qualities of raw milk of buffalo and Sheep for three animal each viz. buffalo and Sheep for ten days as replication different parameter were subject to statistical analysis applying the technique of analysis of variance (f-test) the most widely used method for determining protein content by kjeldahi method for nitrogen determination since nitrogen is a characteristic can be finding. In view of the finding and results presented above, it may be concluded that the chemical quality of milk of buffalo was superior than Sheep milk, due to higher protein, specific gravity, fat content, lactose, total solid and solid not fat, and lower ash and water content in Sheep milk.

Key Words : *Raw milk, chemical quality, buffalo, sheep*

INTRODUCTON

Raw milk has not been pasteurized or homogenized. It primarily comes from cows but also goats, sheep, buffalos or even camels. It can be used to make a variety of products, including cheese, yogurt and ice cream. An estimated 3.4% of Americans drink raw milk regularly (Caroline Hill and M Hum Nutr, 2018). Buffalo milk is very white and beautifully smooth, it differs considerablyin composition from cow's milk. Buffalo milk contains more fat, protein,calcium and phosphorus than cow's milk. The high milk solids of cow andbuffalo milk make it ideal for processing into dairy products, (Abd Elsalam et al., 1982 and Hofi et al., 1982).

Milk is important part of human life. It contains minerals those play a vitalrole in milk uses human consumption. Since milk is generally viewed asnutritious food with lots of vitamins, minerals and fats, proteins etc. thusused for drinking. It contains minerals those play prophylactic role in cancer, auto immune diseases, heart diseases etc.Minerals play a vital role in milkused for human consumption. Since

milk is generally viewed as nutritious food with lots of vitamins, minerals, fats, proteins etc. thus used for drinking purpose. There are different sources of milk samples available, how ever sufficient information regarding their mineral present, especially protein, fatetc. Milk is processed into a variety of dairy products such as cream, butter, yogurt, kefir, ice cream, and cheese. Modern industrial processes use milk toproduce casein, whey protein, lactose, condensed milk, powdered milk, andmany other food-additives and industrial products. Comparative study between the different types of milk is not available much so present studywas carried out to compare the Buffalo milk and Cow milk samples containing reducing sugar, solid not fat and to check the quality of milk, (Dadasaheb Navale, and Shelley Gupta 2016).

Buffalo milk (BM) plays an important role in human nutrition particularly in the developing countries. Compared with CM, buffalo milk is richer in almost all the main milk nutrients. Also, some milk products such as Mozzarella cheese and ghee are the specialties of buffalo milk. In addition, a recent study (Sheehan and Phipatanakul 2009) indicated that subjects with CM allergies are capable of tolerating BM, thus adding to the nutritional benefits of buffalo milk. The composition, properties and processing of buffalo milk and milk products has been the subject of several reviews (Laxminaryana and Dastur 1968; Abd El-Salam 1990; Gokhale et al., 2001; Pandya et. al., 2004).

Generally, sheep milk production is concentrated on cheese manufacture, usuallyconducted atfarm level or in small local dairies. Composition characteristics favorsheep milk for cheese production. Over the years, Brazilian legislation hadstipulated technical regulations about the identity and quality of milk and dairyproducts, however, up to date no specific legislation has been established for theidentity and quality of sheep milk in Brazil. Recently, Normative Instruction 62stipulated updated values of SCC and microbiological counts for milk, as well asphysical-chemical parameters, applicable only to cow milk. (Brasil. Ministério da Agriculture and Pecuária e Abastecimento, 2011)

MATERIALS AND METHODS

DURATION AND PLACE OF STUDY-

The period of experiment was (January - February 2021). Milk was collected at the Mini Dairy Farm Rajaula Livestock Production and Management (Unit), Department of Natural resource management (NRM), Faculty of Agriculture, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot–Satna (Madhya Pradesh).

COLLECTION OF SAMPLE

The objective was to find out the comparative chemical qualities of raw milk of Buffalo and sheep for three animal each viz. Buffalo and sheep for ten days as replication different parameter were subject to statistical analysis applying the technique of analysis of variance (f-test) the most widely used method for determining protein content by kjeldahl method for nitrogen determination since nitrogen is a characteristic can be finding.

Distribution of buffalo and Sheeps.

Buffalos no. :60,70,80.

Sheeps no. :112,113,114.

Cow			**Sheep**		
C_1	C_2	C_3	S_1	S_2	S_3
60	70	80	112	113	114

Determination of Protein

Calculate the nitrogen content, expressed as a percentage by mass, by following formula-

$$Wn = \frac{1.4007 \text{ X } (Vs\text{-}Vb) \text{ X } N}{W}$$

Wn = nitrogen content of sample, expressed as a percentage by mass;

VS = Volume in ml of the standard hydrochloric acid used for sample;

VB = Volume in ml of the standard hydrochloric acid used for blank test;

N = Normality of the standard hydrochloric acid expressed to four decimal places;

W = mass of test portioning , expressed to nearest0.1mg.

Determination of Ash

$$\text{Total Ash} = \frac{(M2-M)}{(100-Mo) \times (M1-M)}.$$

Where,

M2= massing, of the crucible with ash;

M= massing, of the empty crucible; and

M1= massing, of the crucible with the material taken for the test;M0 = moisture, % by mass, calculated as per the method for dried milk.

Determination of total solid

$$\text{Total Solids \% by mass} = \frac{(M2-M)}{X100\ (M1-M)}.$$

Where, M= massing, of the dish, lid and stirring rod;

M1= massing, of the dish, lid, stirring rod and test portion; and

M2= ing, of the dish, lid, stirring rod and dried test portion.

Determination of water:-

Water percent

Water percent = 100-T.S.

Where,

T.S.= Total Solids

Determination Solid Not Fat

$$\frac{\text{volume of 0.1NaOHx100x100x10}}{\text{Weight of MSNF x Weight of sample}}$$

RESULTS AND DISCUSSION

(1) Protein (%)

Table -1 furnish the data on protein percentage in

Table - 1 : Profein (%) in Buffalo and Sheep milk

Sl. No.	Replica-tion	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	3.90	4.00	3.90	**3.93**	4.35	4.45	4.50	**4.43**
2	R_2	3.80	3.80	3.70	**3.77**	4.45	4.45	4.50	**4.47**
3	R_3	4.00	4.10	4.00	**4.03**	4.40	4.45	4.60	**4.48**
4	R_4	4.20	4.30	4.20	**4.23**	4.35	4.45	4.50	**4.43**
5	R_5	4.40	4.50	4.40	**4.43**	4.35	4.45	4.50	**4.43**
6	R_6	4.55	4.60	4.50	**4.55**	4.40	4.45	4.50	**4.45**
7	R_7	4.35	4.40	4.30	**4.35**	4.40	4.40	4.50	**4.43**
8	R_8	4.50	4.60	4.50	**4.53**	4.40	4.50	4.60	**4.50**
9	R_9	3.60	3.60	3.50	**3.57**	4.35	4.50	4.60	**4.48**
10	R_{10}	3.85	3.90	3.80	**3.85**	4.45	4.45	4.60	**4.50**
Range	Minimum	3.60	3.60	3.50		4.35	4.40	4.50	
	Maximum	4.55	4.60	4.50		4.45	4.50	4.60	
	Mean	**4.12**	**4.18**	**4.08**	**4.13**	**4.39**	**4.46**	**4.54**	**4.46**
F- test					S				S
S. Ed. (±)					0.02				0.03
C. D. (P = 0.05)					0.04				0.06

raw milk of Buffalo and Sheep. The results obtained showed that Buffalo and Sheep registered mean protein percentage as 4.12, 4.18, 4.08 (overall 4.13) and 4.39, 4.46, 4.54 (overall 4.46), respectively. The differences in the values due to three animals each were found significant, but due to replication, the difference was significant in Buffalo milk and non-significant in Sheep milk.

(2) Specific gravity (%)

Table 2 contain the data on specific gravity (cc) of raw milk of Buffalo and Sheep. The results obtained showed that Buffalo and Sheep registered mean specific gravity as 1.107, 1.113, 1.104 (overall 1.108), and 1.148, 1.145, 1.155 (overall 1.149 cc), respectively. The differences in the values due to three animals each, were found significant. Due to Replication also, the differences were non-significant.

Table - 2 : Specific gravity (cc) of Buffalo and Sheep milk

Sl. No.	Replication	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	1.110	1.100	1.100	**1.103**	1.155	1.145	1.135	**1.145**
2	R_2	1.115	1.120	1.120	**1.118**	1.160	1.125	1.160	**1.148**
3	R_3	1.100	1.120	1.080	**1.100**	1.155	1.155	1.165	**1.158**
4	R_4	1.105	1.120	1.100	**1.108**	1.135	1.150	1.155	**1.147**
5	R_5	1.105	1.110	1.105	**1.107**	1.110	1.155	1.165	**1.143**
6	R_6	1.120	1.130	1.105	**1.118**	1.155	1.140	1.155	**1.150**
7	R_7	1.105	1.115	1.110	**1.110**	1.160	1.155	1.155	**1.157**
8	R_8	1.100	1.100	1.095	**1.098**	1.140	1.150	1.140	**1.143**
9	R_9	1.100	1.100	1.120	**1.107**	1.155	1.135	1.165	**1.152**
10	R_{10}	1.110	1.115	1.100	**1.108**	1.150	1.135	1.155	**1.147**
Range	Minimum	1.100	1.100	1.080		1.110	1.125	1.135	
	Maximum	1.120	1.130	1.120		1.160	1.155	1.165	
	Mean	**1.107**	**1.113**	**1.104**	**1.108**	**1.148**	**1.145**	**1.155**	**1.149**
F- test					**NS**				**NS**
S. Ed. (±)					-				-
C. D. (P = 0.05)					-				-

(3) Fat (%)

The data on fat percentage in raw milk of Buffalo and Sheep are furnished in Table 3.0 The results contained in the Table showed that Buffalo and Sheep registered mean fat percentage as 6.02, 5.96, 5.89 (overall 5.95) and 8.31, 8.25, 8.28 (overall 8.28), respectively. The differences in these values due to three animals each, as well as due to replication, were significant. Higher mean fat percentage (8.28) was recorded in the milk of Sheep followed by Buffalo (5.95).

Table - 3 : Fat (%) in Buffalo and Sheep milk

Sl. No.	Replica-tion	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	6.30	6.25	6.10	**6.22**	8.30	8.25	8.35	**8.30**
2	R_2	5.80	5.70	5.70	**5.73**	8.20	8.25	8.10	**8.18**
3	R_3	5.75	5.65	5.60	**5.67**	8.35	8.25	8.25	**8.28**
4	R_4	6.05	6.00	5.90	**5.98**	8.30	8.25	8.25	**8.27**
5	R_5	6.00	6.00	5.95	**5.98**	8.30	8.25	8.30	**8.28**
6	R_6	5.85	5.75	5.70	**5.77**	8.35	8.25	8.35	**8.32**
7	R_7	6.55	6.60	6.50	**6.55**	8.35	8.25	8.30	**8.30**
8	R_8	6.20	6.10	6.00	**6.10**	8.30	8.20	8.30	**8.27**
9	R_9	6.15	6.05	6.00	**6.07**	8.30	8.20	8.30	**8.27**
10	R_{10}	5.50	5.50	5.40	**5.47**	8.35	8.30	8.30	**8.32**
Range	Minimum	5.50	5.50	5.40		8.20	8.20	8.10	
	Maximum	6.55	6.60	6.50		8.35	8.30	8.35	
	Mean	**6.02**	**5.96**	**5.89**	**5.95**	**8.31**	**8.25**	**8.28**	**8.28**
F- test					S				S
S. Ed. (±)					0.03				0.03
C. D. (P = 0.05)					0.06				0.07

(4) Lactose (%)

Table 4.0 presents the data on lactose percentage in raw milk of Buffalo and Sheep. The results contained in the Table showed that Buffalo and Sheep registered mean lactose percentage as 4.31, 4.19, 4.07 (overall 4.19) and 4.69, 4.56, 4.66 (overall 4.63), respectively. Lactose percentage was obtained higher in Sheep milk (4.63) as compared to Buffalo milk (4.19). The differences in the values due to three animals each, as well as due to replication, were found significant.

(5) Ash (%)

Table 5.0 presents the data on ash percentage in raw milk of Buffalo and Sheep. The results contained in the Table showed that Buffalo and Sheep registered mean ash percentage as 0.74, 0.71, 0.68 (overall 0.71) and 0.64, 0.67, 0.65 (overall 0.65), respectively. The differences in these values due to three animals each, as well as due to replication, were non-significant. Ash percentage was lower in Sheep milk as compared to Buffalo milk.

(6) Total solid (%)

The data on total solid percentage in raw milk of Buffalo and Sheep are furnished in Table 6.0. The results contained in the Table showed that Buffalo and Sheep registered mean total solid percentage as 16.19, 16.11, 16.08 (overall 16.12) and 17.40, 17.57, 17.74 (overall 17.57) respectively. The differences in these values due to three animals each, as well as due to replication, were significant. Percentage of total solid was higher in the milk of Sheep as compared to Buffalo milk.

Table - 4 : Lactose (%) in Buffalo and Sheep milk

Sl. No.	Replication	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	4.20	4.10	4.00	**4.10**	4.70	4.60	4.70	**4.67**
2	R_2	4.45	4.35	4.25	**4.35**	4.60	4.70	4.60	**4.63**
3	R_3	4.35	4.20	4.20	**4.25**	4.70	4.60	4.70	**4.67**
4	R_4	4.20	4.05	4.10	**4.12**	4.70	4.50	4.70	**4.63**
5	R_5	4.45	4.20	4.20	**4.28**	4.50	4.35	4.50	**4.45**
6	R_6	4.25	4.00	4.00	**4.08**	4.70	4.55	4.70	**4.65**
7	R_7	4.40	4.30	4.00	**4.23**	4.80	4.70	4.80	**4.77**
8	R_8	4.50	4.40	4.20	**4.37**	4.65	4.50	4.55	**4.57**
9	R_9	4.35	4.25	4.00	**4.20**	4.70	4.50	4.70	**4.63**
10	R_{10}	3.95	4.00	3.75	**3.90**	4.80	4.60	4.60	**4.67**
Range	Minimum	3.95	4.00	3.75		4.50	4.35	4.50	
	Maximum	4.50	4.40	4.25		4.80	4.70	4.80	
	Mean	**4.31**	**4.19**	**4.07**	**4.19**	**4.69**	**4.56**	**4.66**	**4.63**
F- test					**S**				**S**
S. Ed. (±)					0.06				0.05
C. D. (P = 0.05)					0.12				0.10

Table - 5 : Ash (%) in Buffalo and Sheep milk

Sl. No.	Replication	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	0.80	0.70	0.60	**0.70**	0.65	0.70	0.65	**0.67**
2	R_2	0.75	0.75	0.75	**0.75**	0.65	0.65	0.70	**0.67**
3	R_3	0.75	0.65	0.75	**0.72**	0.65	0.65	0.70	**0.67**
4	R_4	0.75	0.75	0.65	**0.72**	0.65	0.70	0.70	**0.68**
5	R_5	0.80	0.75	0.75	**0.77**	0.65	0.65	0.60	**0.63**
6	R_6	0.70	0.75	0.75	**0.73**	0.60	0.70	0.70	**0.67**
7	R_7	0.75	0.65	0.70	**0.70**	0.60	0.60	0.55	**0.58**
8	R_8	0.70	0.75	0.65	**0.70**	0.65	0.70	0.60	**0.65**
9	R_9	0.70	0.70	0.60	**0.67**	0.70	0.65	0.60	**0.65**
10	R_{10}	0.65	0.60	0.60	**0.62**	0.60	0.65	0.70	**0.65**
Range	Minimum	0.65	0.60	0.60		0.60	0.60	0.55	
	Maximum	0.80	0.75	0.75		0.70	0.70	0.70	
	Mean	**0.74**	**0.71**	**0.68**	**0.71**	**0.64**	**0.67**	**0.65**	**0.65**
F- test					**NS**				**NS**
S. Ed. (±)					-				-
C. D. (P = 0.05)					-				-

Table - 6 : Total Solid (%) in Buffalo and Sheep milk

Sl. No.	Replica-tion	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	16.15	16.00	16.00	**16.05**	17.50	17.65	17.80	**17.65**
2	R_2	17.00	16.85	16.85	**16.90**	17.30	17.35	17.60	**17.42**
3	R_3	16.25	16.10	16.10	**16.15**	17.30	17.30	17.60	**17.40**
4	R_4	17.10	17.00	17.00	**17.03**	17.40	17.65	17.60	**17.55**
5	R_5	17.00	16.95	16.90	**16.95**	17.40	17.80	17.70	**17.63**
6	R_6	15.90	15.80	15.70	**15.80**	17.50	17.80	18.00	**17.77**
7	R_7	15.60	15.60	15.55	**15.58**	17.40	17.45	17.65	**17.50**
8	R_8	15.00	15.00	15.00	**15.00**	17.60	17.70	18.00	**17.77**
9	R_9	16.25	16.25	16.10	**16.20**	17.20	17.50	17.70	**17.47**
10	R_{10}	15.60	15.50	15.60	**15.57**	17.40	17.45	17.70	**17.52**
Range	Minimum	15.00	15.00	15.00		17.20	17.30	17.60	
	Maximum	17.10	17.00	17.00		17.60	17.80	18.00	
	Mean	**16.19**	**16.11**	**16.08**	**16.12**	**17.40**	**17.57**	**17.74**	**17.57**
F- test					S				S
S. Ed. (±)					0.04				0.07
C. D. (P = 0.05)					0.08				0.15

(7) Water (%)

The data on water percentage in raw milk of Buffalo and Sheep are furnished in Table 7.0. The results contained in the Table showed that Buffalo and Sheep registered mean water percentage as 83.82, 83.90, 83.92 (overall 83.88) and 82.60, 82.44, 82.27 (overall 82.43) respectively. The differences in these values due to three animals each, as well as due to replication, were significant. Water content was found lower in Sheep milk as compared to Buffalo milk.

(8) Solid not fat (SNF) (%)

Table 8.0 shows the data on SNF percentage in raw milk of Buffalo and Sheep. The results contained in the Table showed that Buffalo and Sheep registered mean SNF percentage as 10.17, 10.09, 10.20 (overall 10.15) and 10.09, 10.32, 10.46 (overall (10.29), respectively. The differences in these values due to animals, as well as due to replication, were significant. SNF percentage was higher in Sheep milk as compared to Buffalo milk.

The results of the investigation regarding the chemical qualities of milk of Buffalo and Sheep, have been presented in tables, graphically represented, and discussed in the preceding chapters.

Results of the experiment are summarized below:

1. Higher protein percentage was recorded in the milk of Sheep as compared to Buffalo milk.
2. Specific gravity of Sheep milk was higher as compared to Buffalo milk.
3. Fat percentage was recorded higher in the

Table - 7 : Water (%) in Buffalo and Sheep milk

Sl. No.	Replication	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	83.85	84.00	84.00	**83.95**	82.50	82.35	82.20	**82.35**
2	R_2	83.00	83.15	83.15	**83.10**	82.70	82.65	82.40	**82.58**
3	R_3	83.75	83.90	83.90	**83.85**	82.70	82.70	82.40	**82.60**
4	R_4	82.90	83.00	83.00	**82.97**	82.60	82.35	82.40	**82.45**
5	R_5	83.00	83.05	83.10	**83.05**	82.60	82.20	82.30	**82.37**
6	R_6	84.10	84.20	84.30	**84.20**	82.50	82.20	82.00	**82.23**
7	R_7	84.40	84.40	84.45	**84.42**	82.60	82.55	82.35	**82.50**
8	R_8	85.00	85.00	85.00	**85.00**	82.40	82.30	82.00	**82.23**
9	R_9	83.75	83.75	83.90	**83.80**	82.80	82.50	82.30	**82.53**
10	R_{10}	84.40	84.50	84.40	**84.43**	82.60	82.55	82.30	**82.48**
Range	Minimum	82.90	83.00	83.00		82.40	82.20	82.00	
	Maximum	85.00	85.00	85.00		82.80	82.70	82.40	
	Mean	**83.82**	**83.90**	**83.92**	**83.88**	**82.60**	**82.44**	**82.27**	**82.43**
F- test					S				S
S. Ed. (±)					0.04				0.07
C. D. (P = 0.05)					0.08				0.15

Table - 8 : Solid not fat (%) (SNF) in Buffalo and Sheep milk

Sl. No.	Replication	Buffalo (B)			Mean	Sheep (S)			Mean
		B_1	B_2	B_3		S_1	S_2	S_3	
1	R_1	9.85	9.75	9.90	**9.83**	10.20	10.40	10.45	**10.35**
2	R_2	11.20	11.15	11.15	**11.17**	10.10	10.10	10.50	**10.23**
3	R_3	10.50	10.15	10.50	**10.38**	9.95	10.05	10.35	**10.12**
4	R_4	11.05	11.00	11.10	**11.05**	10.10	10.40	10.35	**10.28**
5	R_5	11.00	10.65	10.95	**10.87**	10.10	10.55	10.40	**10.35**
6	R_6	10.05	10.05	10.00	**10.03**	10.15	10.55	10.65	**10.45**
7	R_7	9.05	9.00	9.05	**9.03**	10.05	10.20	10.35	**10.20**
8	R_8	8.80	8.90	9.00	**8.90**	10.30	10.50	10.70	**10.50**
9	R_9	10.10	10.20	10.10	**10.13**	9.90	10.30	10.40	**10.20**
10	R_{10}	10.10	10.00	10.20	**10.10**	10.05	10.15	10.40	**10.20**
Range	Minimum	8.80	8.90	9.00		9.90	10.05	10.35	
	Maximum	11.20	11.15	11.15		10.30	10.55	10.70	
	Mean	**10.17**	**10.09**	**10.20**	**10.15**	**10.09**	**10.32**	**10.46**	**10.29**
F- test					S				S
S. Ed. (±)					0.08				0.08
C. D. (P = 0.05)					0.16				0.17

milk of Sheep followed by Buffalo milk.

4. Milk of Sheep recorded higher lactose percentage in comparison to Buffalo milk.
5. Lower ash percentage was found in the milk of Sheep compared to Buffalo milk.
6. Total solid percentage in Sheep milk was found higher than that in Buffalo milk.
7. Water content was recorded lower in the milk Sheep as compared to Buffalo milk.
8. Solid not fat (SNF) was found higher in Sheep milk followed by Buffalo milk.
9. Based on the above results, chemical quality of Sheep milk was found superior than Buffalo milk.

CONCLUSION

In view of the findings and results presented above, it may be concluded that with higher Protein, Specific Gravity, Fat, Lactose, Total Solid and Fat content; and lower Ash and Water content, the chemical quality of milk of Sheep was superior to Buffalo milk.

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STUDIES ON FOOD TECHNOLOGY OF GUAVA FRUITS (PSIDIUM GUAJAVA L.) VARIETIES : ALLAHABAD SAFEDA, APPLE COLOUR AND SARDAR GUAVA

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ABSTRACT

Recipe containing 10 per cent pulp, 12 per cent total soluble solids and 0.25 per cent acidity was found better for preparation of R.T.S., Recipe containing 25 per cent acidity was found ideal for preparation of guava squash.Pulp and sugar in ratio of 1 : ¾ with pH 3.2 was found as ideal recipe for preparation of guava jelly. A recipe containing 50, 35, 5, 7.5 and 2.5 per cent pulp, sugar, glucose, skimmed milk and butter respectively was found ideal for preparation of toffee of guava. A recipe containing 45, 51, 3.75, 0.15 and 0.10 per cent pulp, sugar, butter, citric acid and salt respectively was found ideal for preparation of guava cheese. Total soluble solids remained constant upto third month in R.T.S. and upto second month in squash. There after it increased with time. In the present studies, the variability of physic-chemical composition of guava varieties were recorded. Diferent varieties and recipes were screened and evaluated to find out the ideal variety and recipe for various products of guava. Storage stability of these products were also tested.

Keywords : *R.T.S., guava, toffee, jelly, squash*

INTRODUCTION

The vailablity of fruit: excellent nutritive value, flavor and medicinal properties of guava fruit show great potential for processing into valuable products. Fruits can be utilized to make products like jelly, jam, cheese, toffee, nectar, R.T.S., squash, beverages, etc., but the recipe, suitability of guava varieties and storage stability for these products may vary. The proper attention has not yet been given on these aspects of guava products.

On an average, guava fruits contain moisture (76.1%), protein (1.5%), fat (0.2%), carbohydrate (14.5%), fiber (6.9%), calcium (0.01%), phosphorus (0.04%), iron (1.00%), vitamin B_1 (30 mg.100) g), riboflavin (30 mg/100g) and vitamin C (299 mg/100g). The nutritive value of guava is comparable with apple, apple fruits exceed guava fruit only in terms of iron (1.77%) and

Vitamin B_1 (120 mg /100 g) content. However, guava fruit contain about 150 times and 6.9 times more vitamin C and fibre respectively (Chattopadhyay, 1996).

Guava cultivation has been extended to varying agro-climatic regions owing to wider adaptability even under marginal soils where many of other fruits can not be grown successfully. It can be grown under wide range of pH (4.5 to 8.2), drought and high temperature conditions. Guava is susceptible to severe frost but it requires a distinct winter for developing good fruit quality. In developing countries like India, life style (living manner) is changing very fast and the fruit products are becoming popular. Processing of guava fruits into quality products would be more nutritious than many synthetic products, which are being produced and sold in enormous quantity in our country at very high prices.There fore, to explore the potential of utilizing this fruit for processing industry, present investigation was undertaken the with the following objectives:

To study the physic-chemical properties of guava fruit.

To evaluate the recipe for quality products of R.T.S., squash, jelly, cheese and toffee.

MATERIALS AND METHODS

A Laboratory experiment entitled "Studies on food technology of guava fruits (Psidium guajava L.)" was conducted at Horticulture farm, Kulbhaskar Ashram Post Graduate College, Prayagrajduring the year, 2020-21. The investigton comprised of eleven sets of experiment laid out in Complete Randomised Desion (C.R.D) and details of each experiment are given below.

(A) EVALUATION OF RECIPE FOR GUAVA R.T.S. AND TOFFEE

R.T.S. of following recipe were prepared, replicated three times and evaluated for their organoleptic quality.

Recipe No. T.S.S.(%)	Pulp (%)	Adjusted	Acidity (%)
1	10	12	0.25
2	10	12	0.30
3	10	13	0.25
4	10	13	0.30
5	10	14	0.25
6	10	14	0.30

Toffee of following recipe were prepared, replicated three times and evaluated for their organoleptic quality.

Recipe No.	Pulp (%)	Sugar (%)	Glucose (%)	Skimmed Milk (%)	Butter (%)
1	50	30	10	7.5	2.5
2	50	35	5	7.5	2.5
3	50	40	0	7.5	2.5

RESULTS AND DISCUSSION

EVALUATION OF RECIPE FOR R.T.S.

The perusal of data contained in Table 4.6, on the organoleptic evaluation of various recipes of guava R.T.S. shows that the highest organoleptic score (8.4) was recorded with recipe containing 10 per cent pulp, 12 per cent total soluble solids and 0.25 per cent acidity. This recipe was significantly higher than other recipes in terms of organoleptic score. However, total soluble solids above 12 per cent and acidity above 0.25 per cent and acidity above 0.25 per cent considerably reduced the organoleptic score, but beyod 13 per cent total soluble solids at both the levels of acidity (0.25 and 0.30 per cent), organoleptic score was less than 7, which was unacceptable.

Table : 1 - Organoleptic quality of guava R.T.S. as influenced by different recoipes

Recipe No.	Pulp (%)	T.S.S. (%)	Acidity (%)	Organoleptic quality	
				Score	Rating
1	10	12	0.25	8.4	Liked very much
2	10	12	0.30	7.7	Liked moderately
3	10	13	0.25	7.6	Liked moderately
4	10	13	0.30	7.3	Liked moderately
5	10	14	0.25	6.7	Liked slightly
6	10	14	0.30	6.5	Liked slightly
C.D. (P= 0.05)				0.30	

EVALUATION OF SUITABLE RECIPE FOR GUAVA TOFFEE

The data recorded on organoleptic quality of recipes of guava toffee are embodied in Table 4.12. Among the various recipes evaluated, the maximum organoleptic score was recorded in recipe containing 50 per cent pulp, 35 per cent sugar, 5 per cent glucose, 7.5 per cent skimmed milk and 2.5 per cent butter. This recipe was significantly superior over other recipes, organoleptically. The sugar content beyond 35 per cent and glucose content beyond 5 per cent reduced the organoleptic quality of the product.

Table : 2 - Organoleptic quality of guava toffee as influenced by different recipes

Recipe No	Pulp (%)	Sugar (%)	Glucose (%)	Skimmed Milk(%)	Butter (%)	Organoleptic quality	
						Score	Rating
1	50	30	10	7.5	2.5	7.5	Liked moderately
2	50	35	5	7.5	2.5	8.4	Liked very much
3	50	40	0	7.5	2.5	6.8	Liked slightly
C.D. (P=0.05)					0.05		

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A COMPARATIVE STUDY OF BACTERIAL QUALITIES OF RAW MILK OF GOAT AND SHEEP

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ABSTRACT

A comparative study of bacterial qualities of raw milk of Goat and Sheep was conducted at Livestock production and management (unit), Department of NRM, faculty of agriculture, MGCGV Chitrakoot–Satna (M.P.) during January to February 2021. All sanitary precaution were followed to produce clean milk . The sample of the raw milk of three animal each were replicated ten time and tested to deyermine the standard plate count (SPC) (10^4), lactic acid bacteria count (LABC) (10^3), lipolytic bacteria count (LBC) (10^2) proteolytic bacteria count (PBC) (10^2) and coliform count in the raw milk . The data obtained for the a foresaid tests were subjected to statitical analysis. The result of the statical analysis showed that the differences in mean values of SPC 10^4,LABC/10^3,LBC/10^2,and PBC,10^2. In view of the finding and result presented above , results that the milk of all the animals was of superior quality , due to low bacteria count and absence of coliform. The bacteria quality of milk of goat was found superior than sheep milk due to minimum bacteria count of SPC , LABC,LBC and PBC; and absence of coliform

Keywords: *Raw milk, bacterial quality, goat and sheep*

INTRODUCTION

Milk is a highly nutritious food that can be obtained from a variety of animal sources such as cows, goats, sheep and buffalo, as well as humans, for human consumption. However, the high nutrient content of these milks, which includes proteins, fats, carbohydrates, vitamins, minerals and essential amino acids , all at a near neutral pH and at a high water activity, provides an ideal environment for the growth of many microorganisms. Some of these nutrients are directly available to all microorganisms, while others are provided following the metabolism of major components by specific populations to release components and metabolites that are used by others (Frank, 1997).

It is generally accepted that the lactic acid bacteria

(LAB), a group of bacteria that ferment lactose to lactate, are a dominant population in bovine, goat, sheep and buffalo milk, prior to pasteurisation. The most common LAB genera in milk include Lactococcus, Lactobacillus, Leuconostoc, Streptococcus and Enterococcus. Psychrotrophic populations, which particularly establish themselves during cold storage, are also a major component and frequently include Pseudomonas and Acinetobacter spp. Other strains of non-LAB genera are also encountered in milk, as well as various yeasts and moulds (Quigley *et al*., 2011).

Goats have small land and initial investment requirements, and their adaptability to harsh climates makes them suitable for landless and marginal farmers. Goat milk is very nutritious andis an acceptable food in several parts of the tropics (Devendra,1999).

Compositions of goat milk vary with diet, breed, individuals, parity, season, feeding, management, environmental conditions, locality, stage of lactation, and health status of the udder (Park *et al.,* 2007) which also affects taste of goat milk. Comparison between composition of goat milk with ofcow and human milk is given in(Anifantakis E. M 1986).

Goat milk contains 3.8% fat, 3.4% protein, 4.1% lactose,0.8% ash, 8.9 % SNF (Park *et al.,* 2007) and 87% water (Iqbal *et al.,* 2008). Goat milk differs from cow or human milk inhaving better digestibility, alkalinity, buffering capacity andcertain therapeutic values in medicine and human nutrition(Haenlein and Caccese,1984; Park and Chukwu, 1989; Park,1994).

Density of goat milk is comparable to that of cow milk,while it has higher speci! gravity, viscosity, titratable acidity,but lower refractive index and freezing point than cow milk(Parkash and Jenness, 1968; Haenlein and Wendorff, 2006).

The freezing point of goat milk is about -0.580°C, viscosity13.4 mP at 27°C, titrable acidity as lactic acid ranges from0.11 to 0.18 per cent (Roy and Vadodaria, 2006). Surfacetension of goat milk is within the range of cow milk (Ju'arezand Ramos, 1986). The mean pH value ranges from 6.5 to 6.9.The curd tension of goat milk is much lower than that of cow milk. The average value with pepsin-HCL test was 36 (Roy and Vadodaria, 2006).

Lipids in goat milk have higher physical characteristics,than in cow milk, but there are variations between different reports (Anifantakis, 1986; Park, 2006). Lipids in goat milk have generally higher physical characteristics than in cow milk (Anifantakis, 1986; Park, 2006).

Milk somatic cell count has been considered as the index of glandular irritation in the mammary gland *(*Morek-Kopec *et al.,* 2009)it has been found that infected glands have a high *Milk somatic cell count* (Leitner *et al.,*2004 *b* & Barrón-Bravoa *et al.,*2013). *milk somatic cell count* is widely used for evaluatingmilk quality and to define milk prices (Kalantzopoulos *et al*., 2004 & Raynal- Ljutovac *et al*.,2005)

Milk somatic cell count in milk from healthy goats is higher than the milk somatic cell count observed in milk from uninfected cowand ewes. Unlike in milk from cows and ewes, Polymorphonuclear leukocytes (PMNLs) PMNLscomprise the major leukocyte type (40–87%) in goats milk. Because the neutrophils act as thefirst line of immunological defense against infections, this could explain why goats are moreresistant to mastitis(Tian *et al.,* 2005*).*

In goats, the physiological factors may account for up to 90% of the variation in milk somatic cell count (Haenlein, 2002 & Raynal-Ljutovac *et al.,* 2007).

Mastitis in sheep has a large impact on milk production. Significant changes in the protein, fat,lactose, among other components, may occur as

well as reduced production levels.(Oliveira., et al 2013).

In dairy goats, fat and protein content and milk yield could be affected by daily variations as aconsequence of the incidence of non-infectious, genetic, environmental and seasonal factors (Raynal-L jutovac *et al.,*2007&Tangorra *et al.,* 2008).

Sheep is an important part of the agribusiness economy of Iraq. Milk and other dairy foods provide rich dietary sources of protein, calcium, potassium, magnesium, and vitamin A in human diets all over the world and are also good sources of carotenoids and tocopherols, significant provitamins and natural antioxidants with several biological functions (Barłowska *et al.*, 2011; Chirlaque, 2011).

Most of the sheep milk produced throughout the world is transformed into cheese (Barłowska *et al.*, 2011). For this reason, when we refer to the quality of sheep milk we are concentrating mainly on its capability to be transformed into high quality dairy products, and to produce high yields of these products from each litter of milk. This is often described as the processing performance of the milk (Benciniand Pulina, 1997). Therefore, the aim of the present study was to evaluate the effect sex of lamb on milk quality during lactation period for ewes.

MATERIALS AND METHODS

DURATION AND PLACE OF STUDY

Theperiod of experiment was (January-February 2021). Milkwere collected at the Mini Dairy Farm Rajaula Livestock Production and Management Department of Natural Resource Management (NRM), Faculty of Agriculture, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot– Satna (Madhya Pradesh),

COLLECTION OF SAMPLE

Samples were collected from the milking pail separately in sterile 250ml conical flasks and plugged aseptically with cotton plug. The samples were brought immediately to laboratory for determination of total viable count as standard plate count (SPC) and their four physiological groups *viz.* lactic acid bacterial count (LABC), proteolytic bacteria count (PBC), lipolytic bacterial count (LBC) and coliform count(cc).

Distribution of Goats and Sheep's.

Goats no. :99,100,101.

Sheep's no. : 399,400,401.

Goat			**Sheep**		
G_1	G_2	G_3	S_1	S_2	S_3
99	100	101	399	400	401

PARAMETERS OF STUDY

Following were the bacterial parameters determined as per method of

Chalmers 1953

i. Standard plate count/ml (SPC) for total bacteria
ii. Lactic acid bacterial count (LABC)
iii. Proteolytic bacterial count (PBC)
iv. Lipolytic bacterial count (LBC)
v. Coliform count (CC)

PREPARATION AND STERILIZATION OF GLASSWARES:

CONICAL FLASKS –

Prior to use all the conical flasks were thoroughly cleaned, dried, plugged with absorbent type cotton and then sterilized in an autoclave at120 0C for an hour.

PIPETTES -

Prior to use all the bacteriological pipettes of 1 ml and 10 ml capacity were immersed in chromic acid solution overnight, washed with tap water and dried. They were wrapped in paper and sterilized in hot air oven at120 0C for an hour.

TEST TUBES –

Test tubes were washed thoroughly with detergent and tap water. Then test tubes were used for preparing 9ml blanks of Ringer's solution for dilution of the sample. They were plugged with sterile absorbent cotton and then sterilized in autoclave at 120 0C at 1.2 kg/cm2 for 20 minutes.

PETRI PLATES -

These were thoroughly washed with detergent then tap water and kept on a clean table in inverted position for drying. Dried plates were wrapped in paper in block of 4 in each. These were sterilized in hot oven at 120 0C for an hour.

PREPARATION OF MEDIA FOR MICROBIAL EXAMINATION OF MILK SAMPLES:

RINGER'S SOLUTION

It was needed for dilution of milk samples in desired ratio be foreplating as per **(Prasad and Neeraj, 2004)**

COMPOSITION;

Sodium chloride (NaCl) - 9g
Potassium chloride (KCl) - 0.42g
Calcium chloride ($CaCl_2$) - 0.24g
Sodium bicarbonate ($NaHCO_3$) -0.20g
Distilled water - 1000 ml
*0.48 in case of hydrated salt, ($CaCl_2.6H_20$)

STANDARD PLATE COUNT (SPC) FOR TOTAL BACTERIAL COMPOSITION:

Nutrient Agar medium
Agar-Agar - 15g
Peptone - 5g
Sodium chloride - 5g
Beef extract - 3g
Distilled water – 1000 ml
pH - 7.2

peptone, sod. Chloride (NaCl) and beef extract were dissolved in 1000mldistilled water and pH was adjusted to 7.2 at 60 0C using Bromothymolblue as indicator. Agar power was dissolved in 900 ml distilled water by steaming for 15 minutes and filtered peptone, NaCl and beef extract were added, then dispensed in to conical flasks, pulgged and sterilized in autoclave at 1.25 kg/cm2 for 20 minutes.

Lactic acid bacterial count (LABC)

LABC was determined in lactose agar medium.

COMPOSITION:

Agar-Agar - 15g
Peptone - 5g
Lactose - 20g
Beef extract - 3g
Andred's indicator – 10 ml
Distilled water – 1000 ml
pH - 7.0
Andred'sindicator- Acid fuschsin (0.05 % aq.soln) (50 mg in 100 mlwater).

PROTEOLYTIC BACTERIAL COUNT (PBC)

PBC was determined in nutrient milk agar medium
Nutrient agar – 1000 ml
Sterilized skim milk – 100 ml

20ml sterilized skim milk was added to200 ml of sterilized nutrient agarin conical flask of 250ml just prior to pouring in petri-plates. After incubation for 24 hours the development of clean hollow zone around the colonies in medium indicated the proteolysis by bacteria.

LIPOLYTIC BACTERIAL COUNT (LBC): DETERMINEDIN NILE BLUE SULPHATE AGAR MEDIUM COMPOSITION:

Nutrient agar- 1000 ml
Melted butter fat – 40 ml
Nile blue sulphate indicator (0.1 % -10mlAqueous solution)
pH - 7.0

Nutrient agar was prepared melted butter fat and Nile blue sulphate indicator was added and placed in 250 ml capacity flasks. The medium was steamed for 30 minutes on each of three successive days for sterilization. At the time of use, medium was shaken

vigorously and emulsifying fat globules. Lipolytic bacteria hydrolysed pink fat globules and produced a bluish colour around the beneath the colonies. The unhydrolysed fat globules appeared pink due to the action of Nile blue sulphate.

COLIFORMS COUNT

Coliformswere determind in Ma Conkey's Bile salt Agar medium**(Chalmers, 1953).**

COMPOSITION:

Sodium glycotaurocholate - 5g
Peptone - 20g
Sodium chloride (NaCl) - 15g
Agar-agar (powder) - 15g
Lactose - 10g
Bromocresol aquous solution - 2.5 mlpurple 1 %
Distilled Water – 1000 ml
pH - 7.2

The sodium taurocholate, peptone and sodium chloride were dissolved in1000 ml. distilled water by steaming for 30 minutes and pH adjusted to7.4 at 60 0C. Then agar-agar powder was dissolved at 100 0C and filtered. Lactose and bromocresol powder purple indicator were added to the filtered solution and then plugged and sterilized as mentioned earlier.

STANDARD PLATE BACTERIAL COUNT (SPC/ml)

The following procedure was used for determination of SPC in milk:

1. Milk samples collected were shaken gently 25 times in back and forth motion on a levelled table, in a time of about 7 seconds.
2. Dilutions of agitated samples of milk were prepared with the help of sterilized 9 ml blank of ringer's solution such as 1: 10, 1: 100, 1: 1000.Care was taken to shake the diluted sample as stated above.
3. Sterilized pipettes were used to measure quantity of 1 ml suitable milk21

1. dilution and transferred to priorly marked sterilized petri plates in duplicates.

4. As the dilution was transferred into the petri dishes, the month of agar flasks were flamed safely and approximately 15 ml of nutrient agar medium was poured into each dish to cover about 3mm deep.
5. Agar medium was mixed with the dilution by gently rotating and tilting the dishes. After agar medium became solid, the plates were inverted and incubated for two days at 37 C.
6. After lactation, those plates were selected which had 30 to 300 colonies and counted with the help of Quebec colony counter. The average number of bacterial count on two plates were determined by multiplying it with dilution factor to determine bacterial number per ml of milk.

LACTATION PERIOD:

The incubation times for various physiological groups of bacteria were as follows:

Type of Bacteria	Temperature degree Celsius	Lactation Period
Standard plate count(SPC)	37	48 hr
Proteolytic bacterial count(PBC)	30	24 hr
Lipolytic bacterial count(LBC)	30	48 hr
Lactic acid bacterial count (LABC)	35	48 hr
Coliforms count (CC)	37	30 hr

Source of variation	d.f.	S.S.	M.S.S.	F.Cal. Value	F.Tab. (5%)	Result N/NS	C.D.
Group of sheep (T)	r-1	SS(r)	SS(r) df	Mss(r) Emss		S/NS	
Replications (R)	t-1	SS(t)	SS(t) df	Mss(t) Emss		S/NS	
Error	(t-1)(r-1)	SS(e)	SS(E)/Df				
Total	(rt-1)						

STATISTICAL ANALYSIS OF DATA:

The data on bacterial parameters of milk will tabulated and subjected to analysis of Technique (ANOVA) in RBD as per method to determine bacterial quality of milk.

ANALYSIS OF VARIANCE (ANOVA) FOR THE DATA:

Where,

R = Replication

T = Treatment

d.f.= Degree of freedom

S.S.= Sum of square

MSS = Mean sum of square

F.Cal. = Calculated value of F

F.Tab = Table value at 5% level of significance

Critical difference was calculated by following:

$$C.D. = \frac{\sqrt{2} \times EMSS}{r} \times t(5\%) \text{ error d. F}$$

Where,

C.D.= Critical difference

EMSS = Error mean sum square

r = No. of replication

d.f.= t value at 5% for error de

RESULTS AND DISCUSSION

(1) Standard plate count/mℓ (SPC x 10^4)

Table - 1 and Fig. 1 shows the data on Standard plate count/mℓ (SPC x 10^4) in raw milk of Goat and Sheep. The results obtained showed that the mean Standard plate count/mℓ (SPC x 10^4) in Goat milk was recorded 215.80, 215.35 and 214.95 with overall mean of 215.37 and the difference between the mean values was significant. The mean Standard plate count/mℓ (SPC x 10^4) in Sheep milk was recorded 200.90, 200.35 and 201.40 with overall mean of 200.88. The differences in these values were found significant due to animals as well as due to replication. SPC was found lower in Goat milk in comparison to Sheep milk.

Table : 1 - Standard plate count/mℓ (SPC x 10^4) in Goat and Sheep Milk

Sl. No.	Replica-tion	Goat (G)			Mean	Sheep (S)			Mean
		G_1	G_2	G_3		S_1	S_2	S_3	
1	R_1	216.50	216.50	216.00	**216.33**	201.50	201.00	202.00	**201.50**
2	R_2	215.00	215.00	214.00	**214.67**	200.50	199.00	202.00	**200.50**
3	R_3	216.00	216.00	215.50	**215.83**	201.00	201.00	202.00	**201.33**
4	R_4	217.00	216.00	215.00	**216.00**	201.00	201.00	202.00	**201.33**
5	R_5	216.00	215.50	216.00	**215.83**	201.00	201.00	201.00	**201.00**
6	R_6	216.50	216.00	215.50	**216.00**	202.00	201.00	202.00	**201.67**
7	R_7	216.00	215.50	215.50	**215.67**	201.50	201.00	202.50	**201.67**
8	R_8	215.00	214.00	214.00	**214.33**	199.50	199.00	200.00	**199.50**
9	R_9	215.00	214.00	214.00	**214.33**	201.00	200.00	200.00	**200.33**
10	R_{10}	215.00	215.00	214.00	**214.67**	200.00	199.50	200.50	**200.00**
Range	Minimum	215.00	214.00	214.00		199.50	199.00	200.00	
	Maximum	217.00	216.50	216.00		202.00	201.00	202.50	
	Mean	**215.80**	**215.35**	**214.95**	**215.37**	**200.90**	**200.35**	**201.40**	**200.88**
F- test					S				S
S. Ed. (±)					0.16				0.22
C. D. (P = 0.05)					0.33				0.46

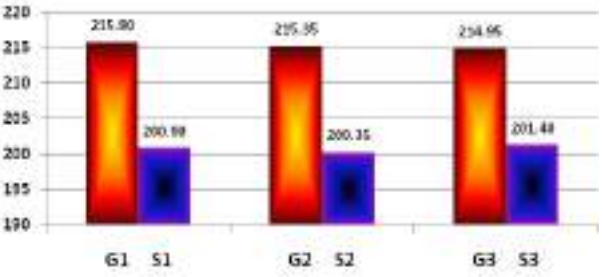

Fig. : 1 - Standard plate count/mℓ (SPC x 10^4) in Goat and Sheep Milk

(2) Lactic acid bacterial count/mℓ (LABC x 10^3)

The data on the Lactic acid bacterial count/mℓ (LABC x 10^3) in raw milk of Goat and Sheep is presented in Table - 2 and Fig. 2. The results obtained showed that the mean Lactic acid bacterial count/mℓ (LABC x 10^3) in Goat milk was recorded 41.90, 41.15 and 41.50 with overall mean of 41.52 and the difference between the mean values was significant. The mean Lactic acid bacterial count/mℓ (LABC x 10^3) in Sheep milk was recorded 38.75, 38.25, and 37.85 with overall mean of 38.28. The differences in these values were found

significant. However, differences in values due to replication were non-significant. LABC was found lower in Sheep milk (38.28) compared to Goat milk (41.52).

Table : 2 - Lactic acid bacterial count/mℓ (LABC x 10^3) in Goat and Sheep milk

Sl. No.	Replica-tion	Goat (G)			Mean	Sheep (S)			Mean
		G_1	G_2	G_3		S_1	S_2	S_3	
1	R_1	42.00	41.50	41.50	**41.67**	39.50	39.00	38.00	**38.83**
2	R_2	41.50	40.50	41.00	**41.00**	39.00	38.00	38.00	**38.33**
3	R_3	41.50	40.50	42.00	**41.33**	39.00	38.50	38.00	**38.50**
4	R_4	42.00	41.50	42.00	**41.83**	39.00	38.00	38.00	**38.33**
5	R_5	42.00	41.00	41.50	**41.50**	38.50	38.00	38.00	**38.17**
6	R_6	41.50	41.00	41.50	**41.33**	39.00	39.00	38.00	**38.67**
7	R_7	42.50	41.50	41.50	**41.83**	38.00	37.50	38.00	**37.83**
8	R_8	42.00	41.50	41.00	**41.50**	38.00	37.50	37.50	**37.67**
9	R_9	42.00	41.00	41.50	**41.50**	39.00	39.00	37.00	**38.33**
10	R_{10}	42.00	41.50	41.50	**41.67**	38.50	38.00	38.00	**38.17**
Range	Minimum	41.50	40.50	41.00		38.00	37.50	37.00	
	Maximum	42.50	41.50	42.00		39.50	39.00	38.00	
	Mean	**41.90**	**41.15**	**41.50**	**41.52**	**38.75**	**38.25**	**37.85**	**38.28**
F- test					S				S
S. Ed. (±)					0.14				0.18
C. D. (P = 0.05)					0.29				0.38

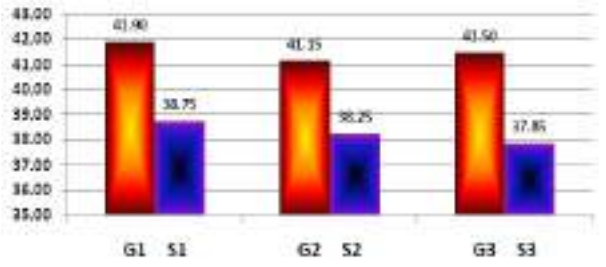

Fig. : 2 - Lactic acid bacterial count/mℓ (LABC x 10^3) in Goat and Sheep Milk

(3) Lipolytic bacterial count/mℓ (LBC x 10^2)

The data on the Lipolytic bacterial count/mℓ (LBC x 10^2) in raw milk of Goat and Sheep are furnished in Table - 3 and Fig. 3. The results obtained showed that the mean Lipolytic bacterial count/mℓ (LBC x 10^2) in Goat milk was recorded 43.15, 42.55 and 42.85 with overall mean of 42.85 and the difference between the mean values was significant. The mean Lipolytic bacterial count/mℓ (LBC x 10^2) in Sheep milk was recorded 39.85, 38.95, and 39.30 with overall mean of 39.37. The differences in these values were found significant. However, differences in values due to replication were non-significant in Goat milk but significant in Sheep milk. LBC was found lower in Sheep milk compared to Goat milk.

Table : 3 - Lipolytic bacterial count/mℓ (LBC x 10^2) in Goat and Sheep milk

Sl. No.	Replica-tion	Goat (G)			Mean	Sheep (S)			Mean
		G_1	G_2	G_3		S_1	S_2	S_3	
1	R_1	43.00	42.50	42.50	**42.67**	39.00	38.50	39.00	**38.83**
2	R_2	43.50	42.50	43.00	**43.00**	39.00	38.00	38.00	**38.33**
3	R_3	43.50	42.50	42.50	**42.83**	40.00	38.50	40.00	**39.50**
4	R_4	43.00	42.50	43.00	**42.83**	39.00	39.00	40.00	**39.33**
5	R_5	43.00	42.50	42.50	**42.67**	40.00	40.00	40.00	**40.00**
6	R_6	43.50	42.50	43.50	**43.17**	40.00	40.00	39.00	**39.67**
7	R_7	43.50	42.50	42.50	**42.83**	40.50	39.00	39.00	**39.50**
8	R_8	43.50	43.00	43.50	**43.33**	40.00	39.00	39.00	**39.33**
9	R_9	42.50	42.00	42.50	**42.33**	41.00	39.00	39.50	**39.83**
10	R_{10}	42.50	43.00	43.00	**42.83**	40.00	38.50	39.50	**39.33**
Range	Minimum	42.50	42.00	42.50		39.00	38.00	38.00	
	Maximum	43.50	43.00	43.50		41.00	40.00	40.00	
	Mean	**43.15**	**42.55**	**42.85**	**42.85**	**39.85**	**38.95**	**39.30**	**39.37**
F- test					S				S
S. Ed. (±)					0.14				0.24
C. D. (P = 0.05)					0.29				0.50

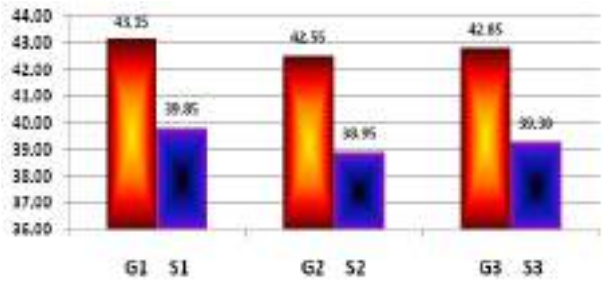

Fig. 3 -Lipolytic bacterial count/mℓ (LBC x 10^2) in Goat and Sheep Milk

(4) Proteolytic bacterial count/mℓ (PBC x 10^2)

The data on the Proteolytic bacterial count/mℓ (PBC x 10^2) in raw milk of Goat and Sheep are shown in Table - 4 and Fig. 4. The results obtained showed that the mean Proteolytic bacterial count/mℓ (PBC x 10^2) in Goat milk was recorded

37.70, 37.15 and 37.40 with overall mean of 37.42 and the difference between the mean values was significant. The mean Proteolytic bacterial count/mℓ (PBC x 10^2) in Sheep milk was recorded 33.70, 33.40 and 33.05 with overall men of 33.38. The differences in these values were found significant. However, differences in values due to replication were non-significant. PBC was found lower in Sheep milk (34.33) compared to Goat milk (37.92).

Table : 4 - Proteolytic bacterial count/mℓ (PBC x 10^2) in Goat and Sheep milk

Sl. No.	Replica-tion	Goat (G)			Mean	Sheep (S)			Mean
		G_1	G_2	G_3		S_1	S_2	S_3	
1	R_1	37.50	37.00	37.00	**37.17**	33.50	33.50	33.00	**33.33**
2	R_2	37.00	37.00	37.50	**37.17**	34.00	34.00	34.00	**34.00**
3	R_3	37.50	37.00	37.50	**37.33**	33.50	33.50	33.00	**33.33**
4	R_4	37.50	37.00	37.50	**37.33**	34.00	33.50	33.00	**33.50**
5	R_5	38.00	37.00	37.50	**37.50**	34.00	33.00	32.50	**33.17**
6	R_6	38.00	37.50	37.50	**37.67**	34.00	33.50	33.00	**33.50**
7	R_7	38.00	37.50	37.50	**37.67**	33.50	33.50	33.00	**33.33**
8	R_8	38.00	37.50	37.00	**37.50**	34.00	33.00	33.00	**33.33**
9	R_9	37.50	37.00	37.50	**37.33**	33.50	33.00	33.00	**33.17**
10	R_{10}	38.00	37.00	37.50	**37.50**	33.00	33.50	33.00	**33.17**
Range	Minimum	37.00	37.00	37.00		33.00	33.00	32.50	
	Maximum	38.00	37.50	37.50		34.00	34.00	34.00	
	Mean	**37.70**	**37.15**	**37.40**	**37.42**	**33.70**	**33.40**	**33.05**	**33.38**
F- test					S				S
S. Ed. (±)					0.11				0.13
C. D. (P = 0.05)					0.24				0.28

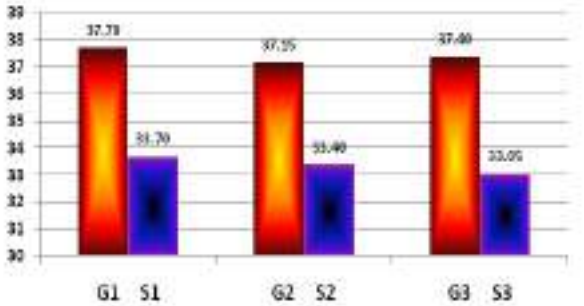

Fig. : 4 - Proteolytic bacterial count/mℓ (PBC x 10^2) in Goat and Sheep Milk

(5) Coliform count/mℓ (CC)

Coliform was not present in any of the samples of Goat and Sheep milk, which indicated that the quality of milk was superior and the management of Dairy Farm was very good.

The results of the investigation regarding the bacterial qualities of milk of Goat and Sheep have been presented in tables, graphically represented, and discussed in the preceding chapters.

Results of the experiment are summarized below:

1. Standard plate count/mℓ (SPC x 10^4) was recorded lower in the milk of Sheep while Goat milk contained higher SPC.
2. Lactic acid bacterial count (LABC x 10^3) was found lowerin the milk of Sheep ,whereas , it was found higher in Goat milk.
3. Milk of Sheep recorded lower Lipolytic bacterial count (LBC x 10^2) in comparison to Goat milk.
4. Lower Proteolytic bacterial count (PBC x 10^2) was recorded in milk of Sheep, where as, the milk of Goat contained higher PBC.
5. Coliform was not found in any of the samples of Goat and Sheep milk, which indicate that the bacterial quality of milk of all the animals was superior and the management activities of the Dairy were good.
6. The differences in values of SPC, LABC, LBC and PBC were significant. Differences in values of SPC& LBC due to replication were significant. The differences in values of LABC, and PBC due to replication were non-significant.
7. Based on the above results, the bacterial quality of milk of Sheep was found superior over Goat milk.

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EFFECT OF ORGANIC MANURES ON GROWTH OF BEET ROOT (BETA VULGARIS L.) C. VLOCAL RED

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ABSTRACT

All the growth parameters of beet root were significantly influenced by the organic manures such as FYM, Vermicompost, Poultry manure.Among different organic manures, sole application of poultry manure (100%) improved plant height at all growth stages. At harvest plant height with T4 PM(100%) recorded highest 34.82 cm and it was at par 30.87 cm with T1 FYM(100%).Maximum number of leaves at harvest was recorded 19.47 leaves with the treatment T6 FYM(50%)+PM(50%) and it was at par 19.00 leaves with T1 Poultry manure (100%).

Keywords : *Beetroot, growth, yield, fertilizer, organic manure.*

INTRODUCTION

For a sustainable crop production system, chemical nutrients removed by the crop must be replenished and physical conditions of the soil is to be maintained organic nutrient management provides excellent opportunities to overcome all the imbalances besides sustaining soil health and enhancing crop production. This optimizes the benefit from all possible sources of plant nutrients in an organic manure. Organic manuring aims in creating a healthy soil , helps in proper energy flows in soil, crop, water, environment while the plant system keeps biological life cycle alive and helps in sustaining considerable level in yield.

Farm yard manure being bulky organic material, releases the soil fertility and soil compactness and improves the aeration in addition to the supply of essential plant nutrients and organic matter and increase soil microbial establishment along with accumulation of excess humus content, It supplies nitrogen, phosphorus and sulphur in available from to the plants through biological decomposition.

Vermicompost provides vital macronutrients [N,P,K,Ca& Mg] and micronutrients [Fe, Mo, Zn and Cu]. Vermicompost besides being a rich source

of micronutrients, also acts as chelating agent and regulates the availability of metallic micronutrients of the plant and increase the plant growth and yield by providing nutrients in the available form. Bone meal is main product of animals raw bone, and has high quantity of phosphorus in organic form for plant growth & other micro nutrients. Poultry manure is an extremely rich source of nitrogen and organic matter. Poultry manures contain 1.0 – 1.8 percent of nitrogen 1.4 - 0 - 1.8percent of phosphorus and 0.8 – 0.9 percent of potassium gurn manuring has a positive influence on the physical and chemical properties of the soil, builds up soil structure and improves tilth, fertility improvement of soils and amelioration of soil problem.

MATERIALS AND METHODS

A field experiment was conducted during rabi season 2020-21 to study the "Effect of organic manures on growth of beetroot {*Betavulgaris* L. } cv. Local Red". Treatments WERE9 and Number of replication were 3. Design of experiment was Randomized Block Design.Total number of plots – 27.

TREATMENT DETAILS :

FYM –Farmyard manure(Dry), VC –Vermicompost, PM – Poultry manure, GM – Green manure, RDF–Recommended dose of fertilizer.

T0	Control unit
T1	FYM(100%) @ 7 t/ha
T2	GM(100%) @ 8.75 t/ha
T3	VC(100%) @ 5.84 t/ha
T4	PM(100%) @ 4.67 t/ha
T5	FYM(50%)@ 3.5t/ha+VC(50%)@ 2.92t/ha
T6	FYM(50%)@ 3.5t/ha + PM(50%)@ 2.33t/ha
T7	FYM(50%)@ 3.5t/ha + GM(50%)@ 4.37t/ha
T8	RDF @ 70kg, 110kg, 70kg (N P K) /ha

RESULTS AND DISCUSSION

1. Plant height (cm) at harvest

The plant height was significantly increased by the application of poultry manure (100%) followed by FYM (50%)+ poultry manure (50%) at different stages of plant growth. The results are presented in Table -1.

Table : 1 - Effect of organic manures on the height of beat root.

No.	Treatments	Plant height(cm) At harvest
T1	FYM (100%)	30.87
T2	GM (100%)	27.67
T3	VC (100%)	29.94
T4	PM (100%)	34.82
T5	FYM (50%)+VC (50%)	28.94
T6	FYM (50%)+PM (50%)	28.67
T7	FYM (50%)+GM (50%)	26.87
T8	RDF@ 70,110,70 (NPK)	25.54
T0	Control	23.67
	CD at 5%	2.48
	SE (m) ±	0.84

At harvest the highest plant height 34.84 cm was recorded in T4 with poultry manures (100%) which was significantly superior to all other treatments RDF T8 recorded a plant height of 25.54 cm. and the lowest was recorded in T0 (23.67cm).

2. Number of Leaves

Number of leaves were significantly affected by the application of organic manures their combination at different stages of plant growth. The results are presented in **Table- 2.**

Table : 2 - Effect of organic manures on the number leaves of beat root.

No.	Treatments	Number of leaves At harvest
T1	FYM (100%)	18.40
T2	GM (100%)	17.90
T3	VC (100%)	18.04
T4	PM (100%)	19.00
T5	FYM (50%)+VC (50%)	17.80
T6	FYM (50%)+PM (50%)	19.47
T7	FYM (50%)+GM (50%)	17.94
T8	RDF@ 70,110,70 (NPK)	18.94
T0	Control	15.70
	CD at 5%	1.76
	SE (m) ±	0.59

At harvest T6 FYM(50%)+PM(50%) recorded indicated highest number of leaves (19.47) which was at par with all the treatments except T4(19.00). The RDF T8 recorded 18.94 cm and Control (15.70) numbers of leaves at harvest.

GROWTH PARAMETERS

The findings pertaining to growth parameters viz height, Number of leaves per plant were observed at harvesting stage. There was significant effect of Organic manures and their combination on all growth parameters. Among the treatments, T5 shows initial germination followed by T4, and T2 .

Plant height at harvest significantly affected by organic manures, Among the treatment T4 PM(100%) recorded the maximum height followed by T6 FYM(50%)+PM(50%), T1 FYM(100%), T3 VC(100%), while the minimum value of plant height was observed in T0 control unit followed by T8 RDF. The positive effect of organic manure on plant height could be due to the contribution made by manure to fertility status of the soil as the soil were low in organic carbon content. Manure when decomposed increased both macro and micro nutrients as well as enhances the physio-chemical properties of the soil. This could have led to its high vegetative growth. The results are in support with findings of Mallangorda *et al* 1995. Manisha 2012.

At harvesting stage number of leaves per plant was influenced by treatment, T6 FYM(50%)+PM(50%) recorded the maximum number of leaves per plant, followed by T4PM(100%) , T8 RDF. However minimum number of leaves per plant was observed with T0 control unit followed by T5 FYM(50%)+VC(50%). Application of organic manures to the soil, physical condition of the soil will be improved by the better aggregation of soil particles Chavan *et al* 1997 Deora, and Singh 2008. Hallmann and Rembialkowaska (2012). These aggregation the soil fertility and often determine the retention and movement of water, diffusion of gases, growth and development of roots in the soil which contributed to the growth of the plant Amon, 1943 Barani and Anburani 2004 By bordiand Malakouti, 2007.

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ECOLOGICAL EFFECT OF INDUSTRIAL AREA IN TWO CITIES OF UTTARAKHAND

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ABSTRACT

A study was carried near the areas of Dehradun and Kotdwara cities of Uttarakhand to evaluate the risk of pollution on water, soil and near industrial area.Water samples were checked for Tota dissolved solids (TDS), Turbidity, pH, Alkalinity, Fluoride, Chloride and Total hardness. The soil sample was checked for pH and number of microorganism. Result were obtained and compared with thecontrol (2kms a part from sampling area). The study concluded that industrial area may be polluted with organic pollutants which resulted in change in some chemical parameters of water mainly total hardness and change in soil pH. Soil sample collected from polluted areas.The polluted area due to increased stress level of microbial diversity was reduced near industrial areas. Sampling and analysis of soil, water is valuable to determine the physio-chemical parameters of the micro environment around the industries. The study concluded that the overall pigment and protein degradation were observed near industrial area and peroxidase activity and pheophytin values were found higher as compared to control, which may be due to temperature variations and presence of pollutants among various parameters of water quality; chloride and TDS was found to be higher around theindustries.

Keywords : *Pollution, biochemical parameters, soilquality, microbialdiversity, turbidity*

INTRODUCTION

Soil is a important and essential elements that shapes the plants life layer as a medium of unconsolidate nutrients and material.soil is a multipart of physical and biological schemes which give support to the plants and supplies essential nutrients to them the process of weathering disintegraterock and transform it into soilnutrients. It forms at hinlayeron surface. It contains mineral sparticles organic matters water and air (lein,2003). Alkalinity, Fluoride, Chloride and soluble potassium. The soil sample was checked for pH and number of microorganism. collected from polluted areas. soil contamination caused by diffuse sources althrough the groundwater was considered to have been without life for a long period. reaserch on the assesments of effects of pharmaceticals waste disposal on bacterial community in soil.theassesment of variation in microbial community structure is of fundamental importance for the evaluation of the impact of an enviromentstresser.

The occurence of soil contamination of natural microbial communities can significantly affects soil moisture organic carbon and potassium have a strong influence on the microbial biomass. The release of pollutants and wastediffers from industry to industry. Fore.g. Leather industry was teismainly composed of chromium zinc, copper, sulphides, carbonates, sodium and many othertoxic organic compounds and inorganic compounds Pulp industry mainly contain carbohydrates ,textile industry containd yes plating industry contain nickel (Nouriet al., 2009). The present study was conducted to determine the impact of industrial pollution on water, soil and vegetation. The study concluded that industrial area may be polluted with organic pollutants which resulted inchange in some chemical parameters of water mainly total hardness and change in soil pH. Soil sample collected from polluted areas. The polluted area due to increased stress level of microbial diversity was reduced near industrial areas. Sampling and analysis of soil, water is valuable to determine thephysio-chemical parameters of the micro environment around the industries. which may be due to temperature variations and presence of pollutants among various parameters of waterquality; chloride and TDS was found to b ehigher around the industries.

The present study was conducted to determine the impact of industrial pollution onwater, soil The waste and pollutants from industries affect soil, water and vegetation equally. There lease of pollutants and waste differs from industry to industry. Fore.g. Leather industry waste is mainly composed of chromium, zinc, copper, sulphides, carbonates, sodium and many other toxicorganic compounds and inorganic compounds Pulp industry mainly contain carbohydrates, textile industry containdyes, plating industry contain nickel (Nourietal., 2009). These pollutants not only alter theq uality of soil and groun dwater but also poseserious problems (Karthikeyanetal. 2010). Microbialactivity in the ecosy stemas they are sensitive to environmental conditions (Wardle 1992; Maithanietal. 1996; Bardgett et al. 1999). They provide precise and immediate information on soil quality. Moreover, the variation in soil microbial biomass afects soil fertility and stability (Bardgettetal. 1999; Angstetal. 2018). Presenceo flargesoil particles reduces the soil moisture content pores and consequently increases with soil organic matterlevel. It is related to soil moisture content, textural class, structure salt content and organic matter. The increase in case of coarse textured soil is larger than that in the fine textured soil. Bulk density of the soil changes with landuse and management practices. Organic matter supplied through the sludge and other kind of waste swhich effect the soil. Schlesinge rand Andrews 2000; Babur and Dindaroglu 2020; Luo et al. 2020; Srivastava et al. 2020; Wu 2020). Few studies also reported that soil biological changes are mostly afected by temperature, moisture and seasonal variations Maithani et al. 1996; Bardgett et al. 1999; Devi and Yadava 2006; Srivastava et al.2020).Seasonality is an important response of any natural ecosystem that has ramifcations over its biodiversity and ecosystem functioning (Tonkinetal. 2017).

MATERIALS AND METHODS

The Study area and sample collection: The study areas were two cities of Uttarakhand, India. First was Dehradun situated between latitudes 29°58' Nand 31°2'N and longitudes 77° 34' E and 78° 18'E and second Kotdwara situated between latitudes 29 °45'0 N and 31°2'N and longitudes 78° 31' 48E. The main industrial area of Dehradun was Selaqui, which is also known as pharmacity as it contains most of the pharmaceutical industries. In Kotdwara the main industrial area is Balbhadrapur, siggadi,j handichaud theregion with three main seasons winter (October–February), summer

(March–June), and rainy (July–September). Soil samples were collected during winter, summer and rainy seasons during 2014–2016 from the temperate forest. soil samples were collected randomly from 0–15 to 15–30 cm soil depths using a soil auger. After removing the litter layer these were mixed to obtain composite samples. The soil samples were sieved (<2mm) to remove stones, pebbles ,root sand plant material and analysed for soil physico-chemical and microbial properties. Soil texture and moisture content were determined by following the Anderson and Ingram (1994). The pH of the soil was measured by using pH meter (Eutech, SN-2069212) with soil water suspension (1:2.5w/vH2O).

Water Sampling :

For water analysis, two sampling sites were chosen one for control and otheras polluted site from both the cities. Water Samples from different sites were collected in the plastic cane of 2.5 litre, about ½ litre water samples was collected from one hand pump from one site and these were mixed to get one sample from one site. In this way sample collected were analyzed in 2-3 days so no special preservation required.

Soil Sampling :

For soil sampling composite sampling was done, where sub-samples were collected from randomly selected locations in a field, and the subsamples are composited for analysis. The soil samples were then air dried and tested in laboratory.

For studying the impact of industrialization on soil and water near the industrial site was chosen and following parameters were compared between control site and industrial site.For water quality analysis around control and industrial site various water parameters like. Total dissolved solids (TDS), Turbidity, pH, Alkalinity, Fluoride, Chloride and Total hardness were analyzed using water testing kit. For assessing the impact of industrialization on soil, soil pH was measured and number of microbial activity determined by plate count method, around control and industrialsite

RESULTS AND DISCUSSION

Source of pollution –The industries in SIDCUL(Kotdwar) region were started in 2013.Nearly 35 industries are established and prosper at the Sigaddi growth center and now they are generating about millions of litres of effluents perday. Appox 70 -80% of effluents are discharge into the soil surface and underwater bodies. The effluents are not onlyrichin waste but also containt oxicmaterials which is dangerous and hazardous toman. The major industries draining effluents into soil surface and ground water bodies. Near SIDCUL kotdwar the iron industries also effects soil surface and soil microbes with their effluents. Physico chemical parameters.

Effect of industrialization on water Quality:

For assessing the quality of water for drinking purpose in these two cities various water parameters were tested and compared with valuesof ISI. The value of pH in control and industrial site of Kotdwara was same but in case of Dehradun pH varied from control to industrial from 7.5 to 6 pH value in both the cities was within desirable limit of 6.5-8.5. The value of pH was in accordance with the alkalinity value, which decreased from control site to industrial site of Dehradun i.e from 200mg/l to 100mg/l and in case of Kotdwara it was 200mg/l in control site and 150 mg/l in industrial site.The desirable limit of TDS is 300mg/l but in both the cities the TDS value was greater than desirable in both control and industrial site. But from control to industrial there was increase of TDS value from 692 mg/L to 750 mg/l in Kotdwara and 698mg/l to 780 mg/l in Dehradun which indicates that increased pollution by extraneous sources can adversely affects the quality of water.The value of Turbidity was 0NTU in both control and industrial which is desirable. The total hardness which is mainly caused

due to calcium and magnesium salts were within the desirable limit of less than 300 ppm. The desirable limit of chloride according to ISI is 250 ppm and in both cities the value of chloride decreased from control to industrialsite.

Effect of industrialization on soil Quality:

For assessing the impact of industrial pollution on soil, the soil pH and microbial growth from the soil sample was analyzed. Soil pH or soil reaction is was found to belower in industrial area of both the cities as compared to controlsite. The soil with pH greater than 8.5 is generally called as sodic soil. But pH of allsoils samples are less than 8.5 indicating that soil samples are free from sodicity hazards. The decreaseinp Hcouldbe due to the decreased amoun to fcarbonate and bicarbonate but overall the pH value neithertoohigh (morethan 8.5) nor too low. The samples were analyzed for microbial growth and it was observed as that the rewas reduction in the growth of microorganisms at different dilutionin both the industrial sites as compared to control site. The pH between 6-8 is favorable for bacterial growth therefore incomparison to fungus bacterialcount was found higher in all the samples. The decrease in number of microorganism both fungus and bacteria near industrial siteas compared to control site may be attributed toaltered pH of soil and water quality condition.

Physico chemical parameters :

The change in soil pH and organic carbon, total nitrogen, total phosphorus and organic matter (percent dry weight basis) contents were determined following standard procedures.The physico –chemical characters like Turbidity and conductivity, pH, temperature, chlorides, Sulphate, nitrates, phosphate and total hardness have increased in the water of the impacted site.

Ecological damage in the vicinity of two Areas.

Table - 1 : Detail of sample location collected from Kotdwara

Sample Source	Sample ID	Latitude	Longitude
1. Under ground Water	KWS (sample)	29. 472124°	78.245518°
2. Under ground water	KWC (control)	29.472806°	78.259603°
3.Soil Sample	KSC (control)	29.472806°	78.259603°
4.Soil Sample	KSS (sample)	29.472124°	78.245518°

Table - 2 : Detail of sample location collected from Dehradun

Sample Source	Sample ID	Latitude	Longitude
1. Under ground Water	DWS (sample)	30.364452°	77.858186°
2. Under ground water	DWC (control)	30. 348341 °	77.890194 °
3.Soil Sample	DSC (control)	30. 348341 °	77.890194 °
4.Soil Sample	DSS (sample)	30.364452°	77.858186°

Table - 3 : Effect of industrialization on water quality

PARAMETER	KOTDWARA		DEHRADUN	
	KWC	KWS	DWC	DWS
TDS (gm)	**0.6924**	**0.7502**	**0.6986**	**0.7894**
Turbidity	**0.0**	**0.0**	**0.0**	**0.0**
pH	**7.0**	**7.0**	**7.6**	**6.6**
Alkalinity (in ppm)	**200**	**150**	**200**	**151**
Fluoride (in ppm)	**0.0**	**0**	**0**	**0**
Chloride (in ppm)	**78**	**115**	**69**	**92**
Total hardness (in ppm)	**300**	**211**	**140**	**175**

Table - 4 : Effect of industrialization on soil pH

Dilution	KOTDWARA		DEHRADUN	
	KSC	KSS	DSC	DSS
1:2	6.0	6.0	6.9	6.3
2:1	7.0	6.1	6.8	6.4
1:1	7.0	6.2	6.8	6.5

Table - 5 : Microbial growth observed for soil sample.

(cfu/g)	KOTDWARA		DEHRADUN	
	DSC	DSS	KSC	KSS
Total bacterial count(10-6)	56	16	700(approx)	175
Totalfungal count(10-6)	3	00	3	0
Total viability (10-6)	59	16	703(approx)	175

CONCLUSION

Sampling and analysis of plants, soil, water is valuable to determine the physio- chemical parameters of the micro environmentar. These changes in plants are biological compensatory responses to environmental stress. Among various parameters of water quality; chloride and TDS was found to be higher around the industries. Talking about ecological study the population density of plants and microbes were found less around industrial sites which shows that there is an impact of industries on population density of organisms and plants. Soil, water and biodiversity are essential elements of ecosystem and are the subject of many agricultural, ecological ,biological and hydrological studies, since large amounts of chemicals enter animal and human food chain through cultivated contaminated soils and water. The study concludes that there is a need to access the ecological risk associated with the polluted areas and necessary actionmustbetakeninthisdirection.

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EFFECT OF INTEGRATED NUTRIENT MANAGEMENT ON GROWTH OF RADISH (RAPHANUS SATIVUS L.) C.V. PUSA DESI

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ABSTRACT

Various treatments showed significant variations in growth parameters such as plant height and number of leaves plant-1of radish. There was a linear increase in plant height at all the stages from 15 DAS to 45 DAS in ascending order. The treatment T6 (1% RDF + 50% (FYM + Poultry manure+ Vermicompost + Neem cake) + PSB + Azotobactor) was recorded significantly maximum plant height. However, the minimum plant height was observed in treatment T8 (Control). The probable reasons for increased plant height may be due to the presence of readily available form of nitrogen through both inorganic and organic sources (Neem cake, FYM, Poultry manure, Vermicompost) where in inorganic source could have exerted positive influence on extended nutrient availability to match the physiological needs of the crop since it is applied in splits, which triggered to produce elevated stature of the growth components. In addition to that integration of Neem cake, FYM, Poultry manure, Vermi-compost might have resulted in beneficial influence of nitrification inhibition and amelioration of soil physical and chemical properties. Each dose of inorganic and organic sources caused significant increase in number of leaves plant-1 from 15 DAS to 45 DAS in ascending order. Significantly maximum leaves plant-1 was observed under treatment T6 (Azotobactor). However, the minimum was observed in treatment T8 (Control). The probable reasons for enhanced number of leaves might be due to promotive effects of macro and micro nutrients from both inorganic and organic sources of nitrogen (Neem cake, FYM, Poultry manure, Vermicompost) on vegetative growth which ultimately lead to more photosynthetic activity. Further, additional amount of phosphorous and other micronutrients such as zinc, copper and iron from Neem cake, Vermicompost might have involved in stimulation of root system through efficient translocation of certain growth stimulating compounds leading to better absorption of nitrogen and other nutrients and their utilization might have improved the number of leaves .

Keywords : *Radish, growth, nutrients, organic munure*

INTRODUCTION

Radish (*Raphanus sativus* L.) belongs to the family Crucifereae. It is a popular root vegetable in both tropical and temperate regions. Probably it is native of Europe or Asia. Radish is grown for its young tender fusiform root.

Organic manures are derived from decayed plant/ animal matters and are free from harmful chemicals. Organic manures are extremely advantageous in enriching soil fertility and do not contain any chemicals which are harmful. Organic manures feed the soil and maintain sustainability in the agro-ecosystem. Growing of crops by the package of organic manures brings forth the organic farming which is in vogue today and organic farming could find a new market scope. Organic farming relies on ecological processes, biodiversity and cycles adapted to the local conditions, rather than the use of inputs with adverse effects. It combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved. There is a heavy demand for this crop throughout the year. Hence yield has to be increased further more. Organic agriculture mainly focuses on utilization of plant residues and manures in agriculture. The organic manuring has positive influence on soil texture towards increased environmental sensitivity, changing food habits, consumers demand for organic food products and supplements are to be considered.

MATERIALS AND METHODS

The experiment was conducted on "Effect of Integrated Nutrient Management on Growth, Yield and Quality of Radish (*Raphanus sativus* L.)" c.v. Pusa Desi was carried out in Rabi season during the year 2020-2021. Experimental designs was Randomized Complete Block Design. Number of treatments **were** 8.Number of replications were 3.

Detail of Treatments:

Treatment Symbol	Treatment Details
T_1	Neem cake (2.5t/ha)+FYM(20t/ha)+PSB(4kg/ha) + Azotobactor (4kg/ha)
T_2	Neem cake (2.5t/ha)+Poultry manure(5t/ha) + PSB(4kg/ha) + Azotobactor(4kg/ha)
T_3	Neem cake (2.5t/ha)+ Vermicompost(5t/ha) + PSB(4kg/ha)+ Azotobactor (4kg/ha)
T_4	Neem cake (2.5t/ha)+PSB(4kg/ha) + Azotobactor(4kg/ha) + 50% FYM
T_5	25% FYM + 25%Poultry manure + 25%Vermicompost + 25%Neem cake + PSB + Azotobactor
T_6	50% Recommended dose of Fertilizers + 50% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + Azotobactor
T_7	75% Recommended dose of Fertilizers + 25%(FYM + Poultry manure + Vermicompost + Neem cake) + PSB + Azotobactor
T_8	RDF (control)

RESULTS AND DISCUSSION

1. Plant height

Plant height of radish as influence by different treatments is given in Table 1. Plant height was recorded at 15,30 and 45 days after sowing. Plant height increased significantly with the increased crop growth period. At 15 days after sowing, the significantly maximum (1 5. 47 cm) plant height was recorded in T_6 (50% RDF + 50% (FYM + poultry manure + vermicompost + neem cake) + PSB + *Azotobactor*), followed by T_7 (75% RDF + 25% (FYM + poultry manure + vermicompost + neem cake) + PSB + *Azotobactor*) (15.19 cm), T_3 (Neem cake 2.5t/ha + Vermicompost 5t/ha + PSB 4kg/ha + *Azotobactor* 4kg/ha) (14.31 cm) and(14.29 cm) and which were at par with each other. While, the minimum (12.59 cm) plant height was observed in treatment T_8 (Control).

As regards to 30 days after sowing, the significantly maximum (32.07 cm) plant height was recorded in T_6 (50% RDF + 50% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) followed by T_7 (75% RDF +

25% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*)
(33.42 cm), T_3 (Neem cake 2.5t/ha + Vermicompost 5t/ha + PSB 4kg/ha + *Azotobactor* 4kg/ha) (34.34 cm), T_1 (Neem cake 2.5t/ha + FYM 20t/ha + PSB 4kg/ha + *Azotobactor* 4kg/ha) (32.21 cm) and which were at Par with each other. While, the minimum (29. 24 cm) plant height was observed in treatment T_8(Control). Subramani *et al.* (2011) and Mani and Anu *et al.* (2018) also draw similar conclusions.

Table-1: Effect of integrated nutrient management on plant height of radish at 15, 30and 45 DAS

Treat. Symb.	*Treatments*	*Plant height (cm) at*		
		15DAS	30DAS	45DAS
T_1	N C(2.5t/ha) +FYM (20t/ha) + PSB (4kg/ha) + Azo.(4kg/ha)	13.57	32.21	33.79
T_2	N C (2.5t/ha) +P M (5t/ha) + PSB (4kg/ha) + Azo.(4kg/ha)	12.71	29.61	31.91
T_3	N C (2.5t/ha) + VC (5t/ha) + PSB (4kg/ha) + Azo.(4kg/ha)	14.31	32.34	34.95
T_4	N C (2.5t/ha) + PSB (4kg/ha) + Azo.(4kg/ha) +50% FYM	13.27	31.75	32.46
T_5	25% FYM + 25% P M + 25% VC + 25% N C + PSB + Azo.	12.81	30.97	32.13
T_6	50% RDF + 50% (FYM + P M + VC + N C) + PSB + Azo.	15.47	34.07	36.15
T_7	75% RDF + 25% (FYM + P M + VC + N C) + PSB + Azo.	15.19	33.42	35.95
T_8	RDF (control)	12.59	39.24	31.52
	S.Em± **(1.102)**			
	C.D. at 5% level **(N/A)**			
	C.V. **(3.433)**			

In case of 45 DAS, treatment T_6 (50% RDF + 50% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*), T_7 (75% RDF + 25% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) and T_3 (Neem cake 2.5t/ha + Vermicompost 5t/ha + PSB 4kg/ha + *Azotobactor* 4kg/ha) were recorded significantly maximum 36.15, 35.95 and 34.95 cm plant height, respectively and which were at par with each other. However, the minimum (31.52cm) plant height was observed in treatment T_8 (Control). findings are in conformity with the findings of Sentiyangla *et al.* (2010), Uddain *et al.* (2010).

2. Number of leaves plant^{-1}

Number of leaves plant^{-1} of different treatments is given in Table .2. Number of leaves plant^{-1} was recorded at 15, 30 and 45 days after sowing.

Table-2: Effect of integrated nutrient management on number of leaves plant^{-1} of radish at 15, 30 and 45 DAS

Treat. Symb.	Treatments	No. of leaves plant^{-1} at		
		15DAS	30DAS	45DAS
T_1	N C(2.5t/ha) +FYM (20t/ha) + PSB (4kg/ha) + Azo.(4kg/ha)	6.21	10.11	11.80
T_2	N C (2.5t/ha) +P M (5t/ha) + PSB (4kg/ha) + Azo.(4kg/ha)	5.97	9.63	11.12
T_3	N C (2.5t/ha) + VC (5t/ha) + PSB (4kg/ha) + Azo.(4kg/ha)	6.48	11.17	12.05
T_4	N C (2.5t/ha) + PSB (4kg/ha) + Azo.(4kg/ha) + 50% FYM	6.17	10.02	11.73
T_5	25% FYM + 25% P M + 25% VC + 25% N C + PSB + Azo.	5.93	9.70	11.27
T_6	50% RDF + 50% (FYM + P M + VC + N C) + PSB + Azo.	6.84	11.84	13.39
T_7	75% RDF + 25% (FYM + P M + VC + N C) + PSB + Azo.	6.83	11.47	12.12
T_8	RDF (control)	5.85	7.93	10.98
	S.Em± **(0.292)**			
	C.D. at 5% level **(0.894)**			
	C.V. **(5.358)**			

Number of leaves plant^{-1} increased significantly with the increased crop growth period. At 15days after sowing, the significantly maximum (6. 84) leaves plant^{-1} was recorded in T_6 (50% RDF + 50% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) at par with T_7 (75% RDF + 25% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) (6.83), while, the minimum (5.85) leaves plant^{-1} was observed in treatment T_8(Control).

In case of 30 DAS, the significantly maximum (11.84) leaves plant^{-1} was recorded in T_6 (50% RDF + 50% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) at par with T_7 (75% RDF + 25% (FYM + poultry manure + vermicompost + neem cake) + PSB + *Azotobactor*) (11.17) and T_3 (Neem cake 2.5t/ha + Vermicompost 5t/ha + PSB 4kg/ha + *Azotobactor* 4kg/ha) (11.67), while, the minimum (7.93) leaves plant^{-1} was observed in treatment T_8 (Control)., Swati Brinjh *et al.* (2014), Khalid *et al.* (2015), Randy (2016) and Mani and Anu *et al.*(2018).

At 45 DAS, significantly maximum 13.49 leaves plant^{-1} was observed under treatment T_6 (50% RDF + 50% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) followed by T_7 (75% RDF + 25% (FYM + Poultry manure + Vermicompost + Neem cake) + PSB + *Azotobactor*) (12.12) and T_3 (Neem cake 2.5t/ha+ Vermicompost 5t/ha + PSB 4 kg/ha + *Azotobactor* 4kg/ha) (12.05) as compared to other treatments. However, the minimum (10.98 leaves plant^{-1}) was observed in t reatment T_8 (Control). Similar results have been reported by Singh *et al*. (2007), Bairwa *et al*. (2009), Uddain *et al*. (2010), Subramani *et al.* (2011).

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EFFECT OF ORGANIC AND INORGANIC FERTILIZERS ON GROWTH OF SPINACH (BETA VULGARIS L.) VAR. PUSA JYOTI

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ABSTRACT

Data regarding on effect of organic and inorganic fertilizers on plant height of Spinach at various stages has been presented in Table 1. Maximum height per plant was produced in the treatment 50% RDF + 50% N through poultry manure (T5) at all stages of observations while minimum height per plant was produced in the treatment control (T1). The data presented in Table 2 revealed that number of leaves of Spinach at various stages. maximum number of leaves per plant was produced in the treatment 50% RDF + 50% N through poultry manure (T5) over the treatments, T2, T6, T7 and T1. However, treatment T1 (control) recorded minimum number of leaves per plant.

Keywords : *Spinach, organic, FYM, poultry manure and vermicompost*

INTRODUCTION

Indian spinach *(Beta vulgaris* L.) is one of the most important leafy vegetable consumed all over the country. It is commonly known as "Palak". It belongs to the family *Chenopodiaceae*, genus *"Beta"* species *vulgaris.* Indian spinach is closely related to Beetroot and Swiss chard. Indian spinach is most probably native of Indo-Chinese region . In India, it is grown on large scale. It is extensively grown in states such as Uttar Pradesh, Punjab, West Bengal, Haryana, Delhi, Madhya Pradesh, Gujrat, Bihar and Maharashtra. Mineral fertilizer decreases both the biological activity in the soil and aggregate stability (Kandeler and Eder, 1990). The intensive use of chemical inputs has not only polluted the soil, water and the environment causing their slow degradation but also affect the life of human being. So, to eliminate all these bad effects organic farming is best alternative. In present conditions is not possible to completely eliminate the use of chemicals especially fertilizers, therefore, use of FYM, compost, city compost, vermicompost.

neemark. poultry manures, press mud, night soil and other organic manures coupled with chemical fertilizers in 21" century, sustainable production is necessary by way of integrated use of nutrients.

MATERIALS AND METHODS

The present study entitled "Effect of organic and inorganic Fertilizers on growth of spinach (*Beta vulgaris* L.) var. Pusa Jyoti", was conducted during *rabi* season of 2020 Department of horticulture Kulbhaskar Ashram Post Graduate College, Prayagraj 211001 (U.P). The details of material used methods adopted during the course of present investigation are summarized below topic wise. The experiment was laid out in randomized block design (RBD) with three replications and seven treatments.

Treatment details

Tr. No.	Treatment
T_1	RDF (control)
T_2	75% RDF + 25 % N through vermicompost
T_3	50 % RDF + 50 % N through vermicompost
T_4	75% RDF + 25 % N through vermicompost
T_5	50% RDF + 50 % N through poultry manure
T_6	75% RDF + 25% N through FYM
T_7	50% RDF + 50% N thorough FYM

RESULTS AND DISCUSSION

1. Plant height

The data presented in **Table-1** clearly showed that the organic fertilizers and their combination played significant role in affecting height of plant. Maximum height per plant was produced in the treatment 50% RDF + 50% N through poultry manure (T_5) at all stages of observations while minimum height per plant was produced in the treatment control (T_1) similar trend was observed during different dates of observation recorded by Gabhiye *et al.* (2003). The better plant height might be due to better development and branching of roots which help in uptake of nutrient as well as more availability of nutrients. These findings are in similar line with the findings of Jat *et al.* (2003).

Table:1- Effect of organic and inorganic fertilizers on mean height (cm) of spinach plant

	Treatment	Height(cm) of spinachplant at				
		30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
T_1	100% RDF(control)	22.60	27.20	25.40	25	25.35
T_2	75%RDF+25%N through vermicompost	23.39	28.12	26.26	26.09	26.52
T_3	50% RDF+50%N through vermicompost	24.65	30.22	28.67	28.37	29.12
T_4	75%RDF+25%N through poultry manure	24.06	29.57	28.38	27.64	28.42
T_5	50%RDF+50%N through poultry manure	25.36	30.72	29.92	28.80	30.20
T_6	75%RDF+25%N through FYM	22.42	27.52	25.54	25.31	24.88
T_7	50% RDF + 50% N	23.62	28.67	26.82	26.43	27.62
	SE +	1.078	1.002	0.9314	1.090	1.102
	CD at 5%	3.266	3.036	2.821	3.303	3.338

2. Number of leaves per plant

Average number of leaves per plant as influenced by different levels of combination of organic manure and inorganic fertilizer were recorded periodically at 30, 45, 60, 75 and 90 days after sowing and are presented in **Table-2** illustration maximum number of leaves per plant was produced in the treatment 50% RDF + 50% N through poultry manure (T5) over the treatments, T2, T6, T7 and T1. However, treatment T1 (control) recorded minimum number of leaves per plant vermicompost and FYM, when applied to soil, plant nutrients are released on large scale and availability of plant nutrient increases which ultimately results in the increase in vegetative growth was justified by Sahu *et al.* (2014). These results are in accordance with Tripathi *et* al. (2013**)** In coriander.

Table -2 : Effect of organic and inorganic fertilizers on mean number of leaves per plant

Tr. No.	Treatment	Mean number of leaves per plant				
		30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
T_1	RDF (Control)	10.05	8.26	8.14	8.62	7.40
T_2	75%RDF+25%N through vermicompost	11.37	8.89	8.27	9.04	7.82
T_3	50% RDF+50%N through vermicompost	13.20	10.65	10.56	11.00	9.32
T_4	75%RDF+25%N through poultry manure	13.01	10.34	10.00	9.98	8.79
T_5	50%RDF+50%N through poultry manure	14.30	11.10	10.90	11.40	9.65
T_6	75%RDF+25%N through FYM	10.12	8.10	7.65	8.60	7.32
T_7	50% RDF + 50% N	12.52	9.17	8.79	9.27	8.36
	SE +	0.473	0.506	0.684	0.52	0.334
	CD at 5%	1.435	1.53	2.07	1.67	1.012

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ROLE OF PHYTOCHEMICAL COMPOUNDS, ESSENTIAL OIL AND IN VITRO SHOOT PROPAGATION OF INTERSPECIFIC F1 HYBRID OF EUCALYPTUS

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ABSTRACT

Eucalyptus is a good source of phytochemical compounds i.e alkaloids, tannins, flavonoids etc extracted from stem, roots and leafs of the tree. The leaves of all the Eucalyptus species contain oil, which is obtained by distillation. The oil is mainly used for medicinal, industrial and perfumery purposes. The tissue culture technology is standardized for its multiplication, using axillary buds of 25-30 years old plants. The axillary buds were surface sterilized with 0.1% Mercuric chloride solution for 10-15 minutes, followed by 0.1% Bavistin treatment for 1 minute and subsequently washed 3-4 times with sterilized distilled water. These surface sterilized axillary buds were cultured on MS medium supplemented with cytokinin and auxin (BAP + NAA). MS medium supplemented with 1.5mg/l BAP + 0.1mg/l NAA proved to be the best hormonal combination for induction of axillary bud which resulted in the development of 1-3 axillary shoots. The proliferated shoots were cultured on MS medium with different concentration of BAP (0.1 – 3.0 mg/l) alone or in combination with NAA (0.1-1.5mg/l) and supplemented with sucrose at 3% level was essential for the development and growth of shoots. These proliferated axillary shoots were excised and subcultured on MS + 1.0 mg/l BAP + 0.1mg/l NAA medium to increase the number of shoots. The aim of this study is to discuss the phytochemical compounds, essential oil and in vitro shoot multiplication of F1 hybrids of Eucalyptus.

Keywords : *Eucalyptus F1 hybrids, phytochemical compounds, essential oil and dmicropropagation,*

INTRODUCTION

Eucalyptus plant is a fast-growing source of timber as well as a source of oil that can be utilized for a variety of reasons. The oil is produced from buds, leaves, stems, fruits, and bark and has antibacterial, antiseptic, antioxidant, anti-inflammatory, and anticancer properties (Dixit *et al.*, 2012, Vecchio *et al*., 2016) making it useful in the treatment of respiratory disorders such as the common cold, influenza, and sinus congestion. The purpose of this paper is to give scientific knowledge regarding phytochemical constituents and *in vitro*

studies, an important aspect of biotechnology has a great potential for rapid and mass multiplication of clonal production of plants. Promising interspecific F_1 hybrids of Eucalyptus developed in India by Forest Research Institute, Dehradun has displayed a very high degree of vigour both in diameter, height and wood quality. Eucalyptus is a control hybrid of *E. tereticornis X E. grandis* (Venkatesh and Sharma, 1979). This hybrid is of immense economic interest because it involves *E. tereticornis* and *E. grandis* as two parent species. The former shows faster growth rate, good stem form, provide best quality of pulp and prefers high rain fall areas while, *E. tereticornis* is drought tolerant species and thus it is very likely that this hybrid may be suited for intermediary zones (Venkatesh and Sharma, 1979).

MATERIALS AND METHODS

Explant source and its culture:

Nodal segments with single axillary bud were used as the source material for micropropagation. The axillary buds were first washed with Cetrimide (ICI ltd. India) solution for 5 min and thereafter surface sterilized with 0.1% Mercuric chloride solution (10-15 min) followed by 1.0% Bavistin treatment for one minute. Other sterilant like $NaOCl_2$(4%) and H_20_2 (20%) were also tested for sterilization of nodal segments. Surface sterilized nodal segments rinsed with 3-4 times sterile distilled water. The surface sterilized axillary buds were cultured on semi-solid Murashige and Skoog's (MS) medium supplemented with cytokinin (BAP and Kinetin). The pH of the medium was adjusted to 5.8 prior to autoclaving the medium at 121° C for 15 min. Cultures were maintained at 25 ± 2 ° C with 16 hrs illumination with the photon flux density of 2500 lux, form white fluorescent tubes.

Establishment and multiplication of shoot cultures:

Axillary bud cultured on liquid and semi-solid MS medium supplemented with cytokinin, resulted in axillary shoots proliferation. These axillary shoots were excised and subcultured on fresh liquid as well as semi-solid MS medium supplemented with BAP (0.1-3.0 mg/l) alone or in combination with NAA (0.1 mg/l – 1.5 mg/l). for further shoot multiplication. Different set of experiments were conducted to obtain the maximum shoot multiplication rate. For this, multiplied shoots were subcultured in a propagule consisting of 6-8 shoots. For each experiment, a minimum of 12 replicates were taken. Observations were recorded after an interval of 5 weeks. Once the optimal shoot multiplication medium was established, the shoots produced were excised in propagules and subcultured every 4-5 weeks. Cultures were multiplied and maintained under 20-30 μEM^{-2} S^{-1} photon flux density for 16 hrs. photoperiod at 25±2°C. The number of propagules cultivated and number of propagules derived at the end of subculture was regarded as the rate of multiplication.

STATISTICAL ANALYSIS

All experiments were repeated thrice. Each treatment consists of 12 replicates. The data representing means of three experiments were analyzed with the help / use of statistical packages viz. Excel ver 2.0 and GenStat ver 8.0 for data of a completely randomized design. The data recorded for various parameters during the study were subjected to one and two way analysis of variance (ANOVA). The significance of the data was ascertained by F-test and the critical difference (C.D.) values at 5% computed, for comparing differences means of various treatments

RESULTS AND DISCUSSION

Phytochemical activity in *Eucalyptus*

Eucalyptus is a good source of phytochemical compounds i.e alkaloids, tannins, flavonoids etc extracted from stem, roots and leafs of the tree. (Dixit *et al.,* 2012). Several researches

were observed to isolate the phytoconstituents from the plant's organs: several volatile constituents as 1,8-cineole (eucalyptol) aromadendrene, ß-pinene, α-0gurjunene, pipertone, globulol, allo-aromadendrene α-,ß-and γ-terpinen-4-ol, were found both in shoots and in leaves (eucalyptol is, in particular, the principal and the most important constituent found in eucalyptus, also in plant's buds); caproic acid, borneol, citral, fenchone, p-menthane, myrtenol, eudesmol, asparagine, myrecene, α-terpineol, glycine, verbinone, cysteine, ornithine, glutamic acid, threonine and ornithine were extracted from fruits (Boulekbache-Makhlouf *et al.,* 2010), while forming acid, sucrose and dextrin were extracted from flowers (Stackpole *et al.,* 2011). Despite the fact that more than 18 compounds were identified in EO, eucalyptol represents the 79.85% of the total chemical composition. The EO also showed a high content of oxygenated monoterpenes, which change between each Eucalyptus species, with a potential variation in therapeutic properties (Olayinka *et al.,* 2012). The composition pattern of essential oil is affected by factors such as geographical location (Usman *et al.,* 2010) and seasons (Emara *et al.,* 2011), with consequent influence on biological activities (Salihu et al., 2011). EO is widely used in many countries like India, China, Portugal, South Africa, Tasmania and Brazil (Emara *et al.,* 2011) for aromatherapy, cosmetic, perfumery, for food and beverages preparation and phytotherapy products (Akolade *et al.,* 2012, Vecchio *et al.,* 2016).

Essential Oil

The popularity of *Eucalyptus* as a plantation species is attributable to their high adaptability, fast growth rate and wide range of uses. The leaves of all the Eucalyptus species contain oil, which is obtained by distillation. The oil is mainly used for medicinal, industrial and perfumery purposes. The most important species in this regard are *E. globulus* and *E. citriodora* the oil of which contain 62% Cineole. Cineole rich oil is used in pharmaceutical, cosmetic preparations and confectionery. It is an active ingredient of inhalant, soaps, mouthwashes, home sprays, tooth pastes, cough and lozenges due to its disinfectant and aromatic qualities. It also finds application as an antiseptic and in mosquito and vermin repellants. Industrial Eucalyptus oil is an useful solvent for varnish, resins, grease, rubber and is used for cleaning paint brushes, greasy hands and removal of stains and for surface coatings of motor vehicles. Eucalyptus oil is used in low perfumes in masking undesired odours. Oil of some species are used as denaturants of alcohol for manufacture of perfumes.

***In vitro* shoot multiplication**

Effect of Phytohormones: The proliferated axillary *in vitro* shoots were excised from the mother explants and cultured on semi-solid MS medium supplemented with 0.1-3.0 mg/l BAP for further *in vitro* shoot multiplication. A high rate of shoot multiplication was obtained due to BAP in the medium, which stimulated the growth of multiple shoots during shoot multiplication cycle. These multiplied *in vitro* shoots were later dissected out into propagule (group of 6-7 shoots) and were subcultured on MS medium supplemented with 0.1-3.0mg/l BAP for further *in vitro* shoot multiplication (Table-1). The best shoot multiplication rate was obtained in MS medium supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA. On this optimal medium the shoot multiplication of 4-5 folds in every 5 weeks subculture duration was obtained (Table-2). MS medium proved to be the best medium for the establishment of shoot cultures in Eucalyptus hybrids. In earlier reports on Eucalyptus F_1 hybrids MS medium has been successfully used for shoot initiation and establishment of Eucalyptus F_1 hybrids cultures (Gupta *et al*., 1981, 1983; Kapoor and Chauhan 1992; Chang *et al.,* 1992;

Table - 1 : Effect of Cytokinin (BAP) on *in vitro* shoot multiplication. MS medium used. Data recorded after 5 weeks.

Hormonal Concentration BAP (mg/l)	Average no. of shoots developed	Multiplication rate	Average no. of shoots length (cm)
Control	11.4 ± 0.93	1.63 ± 0.13	0.70 ± 0.06
0.1	10.6 ± 0.93	1.51 ± 0.13	0.78 ± 0.08
1.0	41.2 ± 1.71	5.89 ± 0.24	1.01 ± 0.08
2.0	34.0 ± 2.54	4.86 ± 0.36	0.76 ± 0.07
3.0	35.2 ± 2.32	5.03 ± 0.62	0.44 ± 0.04
Significance	***	***	***
CD	7.23	1.06	0.20

NS – Non-Significant, *- Significance at 5% **- Significance at 1% ***-Significance at 0.1%

Table - 2 : Effect of hormonal interaction (BAP+NAA) on *in vitro* shoot multiplication. MS medium used. Data recorded after 5 weeks.

Hormonal conc. (mg/l)	Average no. of shoots developed	Multiplication rate	Average no. of shoots length (cm)
0.1 NAA + 1.0 BAP	49.8 ± 1.9	7.12 ± 0.28	1.12 ± 0.03
0.5 NAA + 1.0 BAP	40.5 ± 1.8	5.79 ± 0.26	1.10 ± 0.04
1.0 NAA + 1.0 BAP	31.8 ± 2.00	4.55 ± 0.44	0.75 ± 0.08
1.5 NAA + 1.0 BAP	27.8 ± 1.7	3.98 ± 0.24	0.73 ± 0.09
Significance	***	***	***
CD	6.48	0.947	0.192

NS – Non-Significant, *- Significance at 5% **- Significance at 1% ***-Significance at 0.1%

Bennett,1994; Bisht *et al*., 2000a and 2000b; Joshi *et al*., 2003) and *Eucalyptus* F1 hybrids (Arya *et al*., 2009).

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