BIOFERTILIZER TECHNOLOGY FOR VEGETABLE CROPS OF TEMPERATE HIMALAYAS

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Background

Biofertilizer are microbial inoculants of natural origin unlike synthetic fertilizers. They have a wide range of applicability and leave no toxic non bio-degradable residues. In last few decades there has been a gradual increase in average use of chemical pesticides and fertilizers in Horticulture and vegetable crops, which is adversely affecting the soil health. Therefore, there is an urgent need of alternative technologies and strategies to minimize synthetic inputs and maximize the crop productivity.

Himachal Pradesh is the only state in the country whose almost 89 % population lives in the rural areas. Agriculture is the main occupation of the people, which provides direct employment to at least 62 % of total workers of the state and contribute at least 10 % to the total state domestic products.

The agro-climatic conditions of the state are congenial for the production of cash crops like apple, kiwi, off-season vegetables etc. The state has reached a plateau in usage of cultivable land and now it is focused to increase the productivity levels of crops through advance technologies. Previously, the state agriculture department was dependent on chemical based inputs to fight pests/diseases and crop production. However, recently the state agriculture department has focused on intensifying the organic agriculture by encouraging the use of microbial based bio-controlling agents and biofertilizers. ICAR has funded project - All India Network Project on Soil Biodiversity Biofertilizers (AINP- SBB) to develop microbial formulations to improve productivity of vegetable crops of temperate Himalayas. Biofertilizers has been developed using the promising local strains and evaluated on various vegetables (cauliflower and pea). The strain *Bacillus pumilus* MK5 was isolated for use in cauliflower and *Rhizobium leguminosarum* selected for pea. The strain MK5 increased the cauliflower yield by about 24 % over RDF and saved up to 31 Kg N and 19 Kg P_2O_5/ha chemical fertilizers. Similarly the biofertilizer *Rhizobium leguminosarum* increased green pod yield of pea by 20 % over RDF and 33 % over farmers' practices. Formulation of *R. leguminosarum* saved 10 Kg N/ha. The technical bulletin highlights step by step protocol of biofertilizer production and evaluation of the products. The technology has been accepted and recommended by the State government as package and practices for the vegetable growers. More than 100 farmers were benefitted by this technology and about Rs 10,000/- revenue generated during 2018-2020.

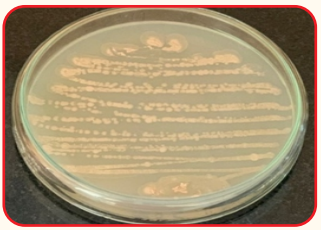
FEATURES OF BIOFERTILIZER:

Characteristics of *Bacillus Pumilus*-MK5	Characteristics of *Rhizobium leguminosarum* strain-CK1
❖ Naturally occurring indigenous isolate ❖ High P-solubilization efficiency (>90%) ❖ High tolerance to carbendazim (1000μg/ml) ❖ Produces plant growth regulators (IAA) ❖ Sequesters iron, siderophore production & multiple antifungal activity ❖ Increased cauliflower yield by 24 % ❖ Can save about 31 Kg N and 19 Kg P_2O_5/ha fertilizers	❖ Naturally occurring indigenous isolate ❖ High P-solubilization efficiency (>95%) ❖ High tolerance to carbendazim (1000μg/ml) ❖ Produces plant growth regulators (IAA) ❖ Sequesters iron, siderophore & multiple antifungal activity ❖ The accretion of available N content can be about 50 Kg/ha

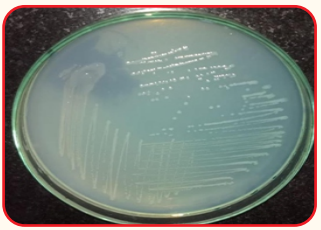
PROTOCOL FOR MOTHER CULTURE PREPARATION

Step 1. Isolation and purification of bacterial isolates

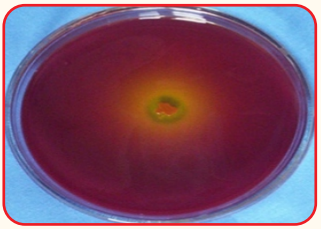
The bacterial strain *Bacillus pumilus* was isolated from rhizosphere of cauliflower by serial plate dilution method on Nutrient Agar medium. The culture of *Rhizobium* spp. was isolated by repeated streak plate method on YEMA medium.

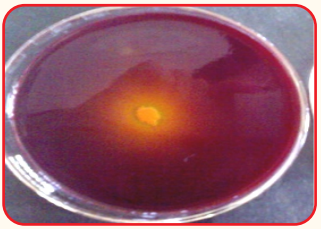


Pure culture of *Bacillus pumilus on* Nutrient Agar medium



Pure culture of *Rhizobium* spp. on YEMA medium

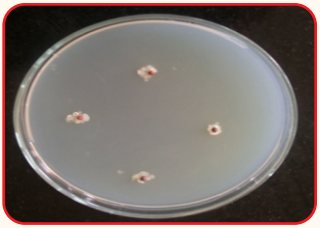




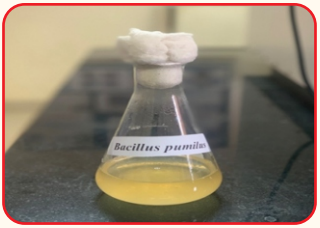
P-solubilization by *Bacillus pumilus* and *Rhizobium* spp. on *Pikovskaya's medium*

Step 2. Bacterization of seeds for screening under laboratory conditions

Cauliflower seeds were surface sterilized with 0.2 per cent mercuric chloride ($HgCl_2$) solution for 5 min and rinsed several (8) times with sterilized distilled water. The surface sterilized seeds were treated with bacterial inoculum and placed on nutrient agar plates. The bacterial growth around the seeds was recorded after 48 h of incubation.



BACTERIZATION OF SEEDS



MOTHER CULTURE

Step 3.Net house studies on efficacy of inoculum

The bacterial formulations with population density of 1.5 OD at 540 nm that resulted in formation of 10^8cfu/ml cells were used for preparation of liquid inoculum. Seeds were soaked in bacterial liquid culture for 3-4 h in sterilized petri plate and thereafter sown in pots under net house conditions.

❖ Pot culture experiment of cauliflower and pea was carried out with possible combinations of bacterial isolates along with different doses of N and P. The experiment was conducted at UHF-Nauni under net house conditions in earthen pots (5 kg capacity) containing mixture of Soil: Farm Yard Manure (FYM): Sand in the ratio of 1:1:1.

❖ A booster dose in liquid form having same optical density was applied at the rate of 10 ml/pot after one month of sowing.

EFFECT OF *Bacillus pumilus* BIOFERTILIZER ON CAULIFLOWER

EFFECT OF *Rhizobium leguminosarum* BIOFERTILIZER ON PEA

The best three bacterial isolates along with three levels of N (Pea) and NP (Cauliflower) were selected from the net house experiments on the basis of various plant growth parameters and soil characteristic at field level evaluation.

LARGE SCALE BIOFERTILIZER PRODUCTION

Step1. The steps involved in production of mother culture of liquid biofertilizer are same as that used for the preparation of quality nursery production.

Pure cultures obtained after serial dilution (*Bacillus* spp.) and repeated streak plate method (*Rhizobium* spp.) were further grown in liquid broths contained in the flasks to achieve population density of 10^8cfu/ml (1.5 O.D. at 540 nm). For mass production of biofertilizers, the mother culture (250ml) prepared was then inoculated in sterilized broth in carboy (15 l capacity) and kept for 4-5 days at room temperature.

LARGE SCALE MULTIPLICATION OF MOTHER CULTURE

Step 2. Field studies on efficacy of inoculum

The demonstration trials were conducted for cauliflower (at variable doses of NP) and pea (at variable doses of N) at different locations. The results are depicted in the Table 1 & 2.

GENERAL VIEW OF FIELD TRIAL

75% NP+*Bacillus pumilus*

GENERAL VIEW OF FIELD TRIAL

60%N+ *Rhizobium leguminosarum*

EFFECT OF BIOFERTILZER ON YIELD OF CAULIFLOWER & PEA

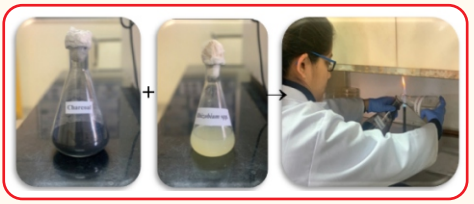
Table 1: Effect of Liquid Biofertilizer on curd yield of cauliflower

Treatments	Curd yield (q/ha)		
	Nauni (Solan)	Kandaghat (Solan)	Ghaluwal (Una)
Recommended package of practices (100% NPK)	196.9	283.6	247.9
MK5+ 75% NP	266.4	356.2	322.2

Table: 2 Effect of Liquid Biofertilizer on green pod yield of pea

Treatments	Pod yield (q/ha)			
	Rekong Peo	Nando/ Nauni	Tikkari	Piran
Recommended package of practices	125.4	123.1	154.3	60.8
Rhizobium leguminosarum+ 60%N	142.4	164.0	181.5	87.7

PREPARATION OF CHARCOAL BASED INOCULUM



200g AUTOCLAVED CHARCOAL+200ml BACTERIAL CELL SUSPENSION (1:1) HAVING 10^8 CFU/ml

CHARCOAL BASED BIOFERTILIZER (*Rhizobium leguminosarum*)

METHOD OF PREPARATION AND APPLICATION OF BIOFERTILIZER

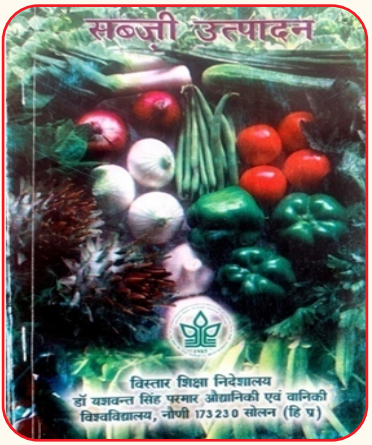
METHOD OF PREPARATION	MODE OF APPLICATION	SUITABILITY	BENEFITS	COST BENEFIT RATIO
CAULIFLOWER Seed treatment : Preparation: 1000ml cell suspension of 1.5 OD at 540nm Seed dipping ratio : - Liquid inoculant: 1000 ml - Cauliflower seeds: 1000 g - Time allowed: 3-4 hours This preparation resulted in coating of $8x10^8$cfu /seed Seedling treatment: Preparation: 2000ml cell suspension of 1.5 OD at 540nm Seeding dipping ratio : - Liquid inoculant : 2000 ml - Cauliflower seedlings : 20,000 Nos. Time allowed: 20-25 min	Treatment of seeds with MK5 liquid inoculum (1000 ml per 1000 g seed) before sowing and seedling dip in 2000 ml inoculum at the time of transplanting along with application of N94, P57 & K72 in place of N125, P76 & K72. Apply whole amount of nitrogen along with other fertilizers at the time of field preparation.	The developed PGPR /bio-fertilizer technology is suitable for different agro-climatic zones of the state as the MK5 isolate is indigenous and compatible with commonly used fungicides for seed treatment	Increased the cauliflower yield by about 24 % Cost effective (B /C ratio= 1:5) Saved 30 Kg N and 20 Kg P_2O_5/ha chemical fertilizers	Application of Liquid bacterial inoculum costs Rs.60 per 1000 ml for 1000g seeds & seedling dipping in 2000ml inoculum for about 20000 Nos. Total Cost Rs 180/- B:C=1:5

PEA Preparation:200 g autoclaved charcoal+200ml cell suspension of 1.5 OD at 540nm The optimum ratio : - Adhesive (20% jaggery) : 1000 ml - Charcoal based inoculant : 200 g - Pea seeds: 10 Kg This preparation resulted in coating of $8x10^8$ cfu /seed	Treatment of pea seed with *R.leguminosarm* charcoal based inoculum (20 g charcoal based culture per kg of seed) before sowing and application of 15 kg ha-1 N in place of 25 kg ha-1 N. Apply whole amount of N along with other fertilizers at the time of field preparation.	The developed bio-fertilizer technology is suitable for different agro-climatic zones of the state as R. *leguminosarm* is indigenous and compatible with commonly used fungicides for seed treatment	Application of biofertilizer *R.leguminosarum* seed treatment plus 60% N increased green pod yield by overall average of 20.80% over RDF and 33.45% over farmers practices, saved 40% (10 Kg N/ha) nitrogen fertilizer.	The application of 60 per cent N + *R.leguminosarum* gave an additional net income of Rs. 26000 ha-1 over control (Without N)& Rs. 3800 ha-1 over 100% N* (1:13 Without N) and (1:7 With 100 % N) *{Average of three years}. (By taking price of Green pea = Rs. 1000 q-1; Fertilizer Rate = Rs. 6 Kg-1; cost of inputs (*Rhizobium* inoculum) = Rs. 200 ha-1 and labour cost = Rs. 200 per day).

TRAININGS / PACKAGE OF PRACTICES/STAKEHOLDERS (2018-20)

BENEFICIARIES	
Farmers benefitted	100 vegetable farmers of the state were benefitted. Dr. KK Bhardwaj, Incharge PCDO Saloh, Tehsil and District Una, 9805011264 Sh. PyaraLal, Village Ghaluwal, Saloh, Tehsil and District Una, 9816431349 Sh.Santokh Kumar, Village Lalsingi, PuranaHoshiarpur Road, Tehsil and District Una, 9817045319 Mr. Sucha Singh, Rakkar Colony, Tehsil and District Una, 9882722866 Mr. Dayaram Sharma, Piran, Village and PO Piran, Tehsil and Distt. Shimla, 9418837908
REVENUE GENERATED (2018-20)	
Revenue Earned (2018-2020)	Rs.10,000 (This includes biofertilizer strains *Rhizobiumlegum inosarum* and *Bacillus pumilus*)

Package of practices	Package of practices developed and recommended to farmers at State level. Package of Practices of vegetable Crop published by Directorate of Extension Education YSP-UHF, Nauni, and Solan (H.P.) 2014 at page No.66 (Pea)



Package of Practices of Vegetable Crop published by Directorate of Extension Education YSP-UHF, Nauni, Solan (H.P.) 2014

The information given in the document is based on the experiments carried out at the Department of Soil Science and Water Management, Dr. YSP-UHF, Nauni, Solan -173230, Himachal Pradesh. For training, demonstration and other enquiries please contact the department.

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