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Effect of Rhizobacterial isolate ALKP 12 on the seed germination and growth pattern of *Hordeum vulgare* L. and its comparison with chemical fertilizer and other treatments

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Abstract : It is generally observed that the productivity of soil and reduction of soil quality has occurred globally due to the excessive use of chemical fertilizer. Alternative methods to improve crop yield without chemical fertilizer is huge challenge worldwide. We have focused our efforts to isolate a plant growth promoter (PGP) from Dehradun, Uttarakhand and isolated a bacterial isolate ALKP12. We investigated this ALKP 12 isolate for PGP activity using *Hordeum vulgare* L. as model plant. After eight days of experiments done in pots in triplicates, we calculated the germination rate, shoot and root height and compared the result with chemical fertilizer, organic manure, water, combination of ALKP 12 and manure and a known bio inoculants. Our results showed that germination rate of seeds of *Hordeum vulgare* was up to 90 percent and averaged 83 ± 3.5 percent which was significantly higher (statistically) when compared to other five treatment groups. The average shoot and root height of the model plant reached up to 34.89 ± 0.59 cm and 10.97 ± 0.873 cm respectively on the 8th day when the seeds were treated with high phosphatase producing isolate ALKP 12. only. This growth was significantly higher when compared with organic manure group that had 11.530 ± 1.056 cm as shoot height and 2.68±0.569 cm as root height for the same day. These result suggest that ALKP 12 can be used as a bio inoculants for increasing growth of *Hordeum vulgare* after further experiments.
Key Words: crop yield, *Hordeum vulagare*, germination rate, shoot height, ALKP 12.

Introduction

Hordeum vulgare L. commonly known as barley is an annual monocotyledonous herb, belonging to the tribe Triticeae, which is evolutionary closely related to two other small grain cereal species, wheat and rye. *Hordeum* genus is known to have diverged 12 million years ago[1]. Today, barley is a significant crop plant globally and it is mainly exploited as feed or as raw material for malt production. In the hilly region of Uttarakhand, farmers cultivate barleyfor the food purpose. Barley is also gaining importance as functional food due to presence of β-glucanase which is useful in lowering the risk of cardio- vascular disease. Other than this, barley has potential application in distilleries (where premium quality whisky is produced), malted milk foods, bakery biscuits, confectionary items, chocolate powders, cornflakes and imitation coffee. Barley is also used in the pharmaceutical industry in production of tonic (starch and maltose syrups), health foods, slim diets, pet food, fish food and medium for bacterial cultivation.. According to the data of [2] Punjab is highest producer of

Barley with productivity of 3364 kg/hectare, followed by Rajasthan with a productivity of 2380 kg/hectare while Uttarakhand has a production of 1375 kg/hectare.

Most agricultural soil have large amounts of inorganic and organic phosphates. These phosphates are immobilized and mostly unavailable. Only a low amount is available to plants and many soil are actually phosphate deficient. High reactivity of phosphates with some metals like iron, alluminium and calcium leads to precipitation or adsorption of about 75%-90% phosphates in soil [3], [4]. More over excessive application of inorganic mineral fertilizer over the last few decades had causes residue toxicity, environmental pollution and soil fertility problem and also altered the structural composition and architecture of the soil [5], [3], [6]. To decrease the use of synthetic agrochemicals in crop production and increasing yield along with maintaining soil fertility and its composition, a need to develop an alternative method has been felt since long. A much desired method should sustain agriculture crop production through minimization of excessive use of inorganic chemical fertilizer. Various scientist all over the world had focused on the plant growth promoting rhizo bacteria and other useful bacteria that could be a substitute to these chemical fertilizers [7].

There are various kinds of soil rhizo bacteria that have been isolated so far and used on the crop plant to increase their growth and crop yield. Some species of Azobacter and Azospirillum have been recommended as biofertilzers for maize mainly grown under tropical region, while a number of other species like *Bacillus* and *pseudomonas* have commonly been recognized for their importance in plant growth and disease. [8], [9].

In our previous study [10] had isolated a microbial isolate, ALKP12, from the district Dehradun, Uttarakhand. This microbe has a unique property of producing high levels of phosphatase enzyme at both acidic and alkaline pH. In the present study we wanted to see whether this ALKP 12 isolate can increase germination rate and growth rate of our plant model *Hordeum vulgare* similar to chemical fertilizer, organic manure and combination of manure along with water and a positive control. We also wanted to see if this isolate could promote the growth of the root and shoot at the initial stage of development.

Materials and Methods

Bacterial isolate ALKP 12, culture conditions and media.

Bacterial isolate ALKP 12 which was stored in 15% glycerol at -80° C for long term storage was thawed and an aliquot was transferred to 500 ml flasks containing nutrient broth [10] and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27°C. The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^9 CFU/ml.

Seed sterilization methods

Hordeum vulagare was used as the model plant for the treatment with ALKP 12 isolate. Seeds were purchased from the local market in the Dehradun district. These were surface sterilized with $HgCl_2$ (0.1g/l) for 10 min **(**Liang et al. 2003**)** and also with 70% ethanol for another 2 min and then soaked in 1.2% sodium hypochlorite for few minute. They were rinsed 10 times thoroughly with sterile deionized double distilled water [11]**.** Plastic pots were used for the experiments and were also sterilized with 20% hypochlorite solution.

Collection of soil samples

Soil samples were taken from the two different agricultural location of Dehradun viz, Doiwala and Mothrawalan and the soil pH was determined in 1:2:5 soil : water suspensions using an automated glass electrode pH meter. The soil was crushed to pass a 2-mm sieve for the sowing experiments [12]**.**

Various treatments groups of model plant – *Hordeum vulgare* L.

Sterilized seeds (thirty four) were sown into four different quadrants of the area pot (diameter of 21 cm) with approximate area of 86 cm^2 each. Quadrants were separated clearly by marking them using a small stick. Six treatments groups were as follows : (a.) Chemical fertilizer (CM) - 0.15 g/ Kg soil. (b.) Organic manure (OM)-0.15 g / Kg soil (c.) Combination of OM with our isolate ALKP 12 (1.2 x 10^7 cfu/ml) (d.) Positive control (PC) that has pseudomonas sp. as inoculum (1.2 x 10^7cfu/ml)and (e.) Control-Water (W) – where 15 ml was poured in each quadrant. This amount of water was used in all of the treatment. (f.) ALKP 12

isolate alone (1.2 x 10^7cfu/ml). In group f, the treatment of barley was done with our isolate ALKP 12. Surface sterilized seeds soaked in a bacterial cell culture broth suspension having the colony forming unit (cfu)/ml- 1.2 x 10^7 for 4 h in a shaker which was adjusted to 60 rpm. After 4 h of soaking of the seeds with the inoculums (ALKP 12), the seeds were removed and dried in the air at room temperature in the laminar air flow. The seeds were then transferred to filter paper and sown on to the soil filled in the plastic pots carefully without the contamination of the hand. For the inorganic chemical fertilizer (CM) treatment, the prepared soil kept in the plastic tray was mixed well in a concentration of 0.15 g Kg^{-1} soil. For the mixture or the amalgam, definite amount (0.15 g Kg^{-1} + 1.2 x 10^7 cfu/ml) was added. For the water treatment , small volume of water was added (15 ml) in to each of the four quadrant where exactly 34 numbers of seedlings were sown. For the positive control similar concentration of isolate load was added. All the treatments were done in the month of April and were again repeated in the next season

Monitoring the seed germination percentage (Vigour index) and calculation of growth pattern of *Hordeum vulgare* seedlings - root and shoot length

All the groups received same amount of water as compared to water only group. The pots were placed in a laboratory where daily photo period was approximately 9 hours and the maximum temperature was approximately 25° C in the day time and at night it was around 18 °C [12]

Germination percentage (G %)/Vigour-index was calculated according to the formula:

G % = (number of germinated grain / total number of grain planted) X 100

Shoot length, root length were recorded every day starting from day 5^{th} till day 8^{th} by removing all the germinated plantlets and then measuring the shoot and root height of each one of the plants in all the three pots using scale

Statistical methods

For every treatments T-test was carried out and we compared the results with our isolate ALKP 12 with other treatments groups.

Results and Discussion

pH of the soil in which the various treatments were carried out was 8.

Germination percentage when calculated after 5^{th} day, was different for all the treatment and data is represented in table 1. High percentage of germination was observed (up-to 90 %) in isolate ALKP 12 alone group and averaged around 83 ± 3.5 (Mean ± S.D) percent. *Hordeum vulgare* in water only treatment group showed next higher percentage of seed germination, up-to 61% and average was 58.75 ± 1.5 percent. Positive control treatment with pseudomonas species should 55 ± 2.87 percent germination while chemical fertilizer treatment group showed 47.5 ± 6.24 percent germination. To our surprise, organic manure along with ALKP 12 treatment group showed considerably less germination i.e. about 36.5 ± 5.74 percent germination. Organic manure alone had the least germination percentage with a mere 20.75 ± 6.18 percent germination. Our result is in accordance with the study conducted by [13] where he also showed that germination rate is less when organic manure was used and it is suggested that this was due to poor supply of essential nutrients like nitrogen and phosphorus. [14] also reported that seed germination can be initiated by water imbibitions and any shortage in water supply will led to seed under stress.

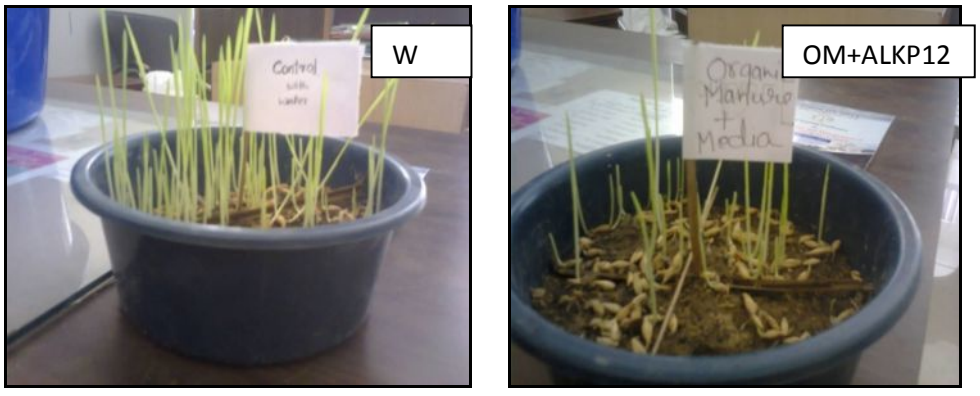
When our bacterial isolate ALKP 12 was used to treat the seedlings of *Hordeum vulgare*, there is a statistically significant increase ($p \leq 0.0003$) in the average length of the shoot that averaged about 20.76 ± 1.60 cm (fig 5a) and average length of root that averaged about 5.25 ± 0.96 cm (Fig 6a) as compared with organic manure on the 5^{th} day. This also suggest that organic manure for the initial growth of barley is not a good option to choose. When we compared to ALKP 12 only group with other treatment groups including the positive control (with Pseudomonas sp.) and water there was a significant difference amongst them. The significance level increased further on 6^{th} day in the average shoot height of barley ($p \leq 0.0006$) and average root height ($p \leq 0.0001$) when ALKP 12 alone treatment was compared with organic manure. Significantly less growth was observed in organic manure treatment for barley. This needs further investigations into the reasons

like insufficient supply of enough phosphorus to the barley, however the experiment for estimating the phosphorus content in the soil before and after the treatment are ongoing. Similarly Fig 5c. shows that there is further rise in the average height of shoot on the 7th day when ALKP 12 alone group which averaged to 30.90 ± 2.06 cms. On 8thday (Fig 5d) there is also, best growth for shoot height was observed in ALKP 12 isolate group averaged to 34.89 ± 0.60 cm as compared with organic manure (Fig 6d , $p \leq 0.0001$). Similar trend was also observed with average root length that also averaged about about 10.97 ± 0.87 cm. Some of our results are in support of [15] where he also observed that when *P.polymyxa* RCO5 was used as bio inoculants, there was an enhanced root and shoot growth but he could not show whether the isolate is a high phosphatase producer. This increase in average the height of shoot and root of *Hordeum vulgarae* may be due to the colonization of this isolate ALKP 12 with the seeds and high intake of phosphate [16] but this needs further investigations.

Table 1: Germination percentage/Vigour index of *Hordeum vulgarae*. Highest seed germination was shown by the ALKP 12 isolate group

Treatments	Ist Quadrant Day 5	2nd Quadrant Day 6	3rd Quadrant Day 7	4th Quadrant Day 8	Average (Mean±. S.D.)
ALKP 12	82	85	88	90	**83 ± 3.5**
OM	17	14	26	26	20.75±6.18.
CM	44	41	50	55	47.5± 6.24..
W	61	58	58	58	58.75± 1.5.
OM+ALKP 12	41	35	41	29	36.5± 5.74
PC	52	55	58	58	55.75± 2.87..

1a **1b**



1c **1d**

1e **1f**

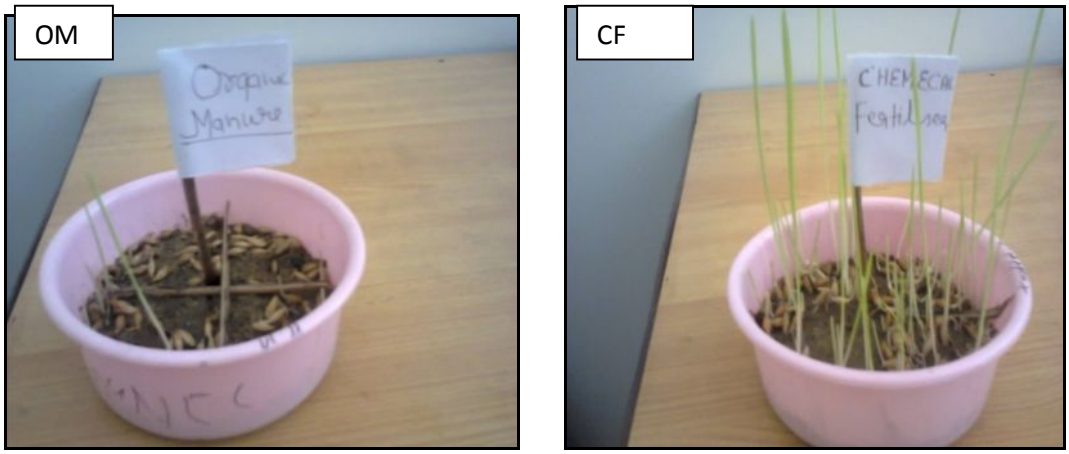


Fig.1. Figures showing effect of various treatments on seeds of *Hordeum vulgare* on germination rate and estimating the average shoot and root height of all the germinated seedlings from day 5 to day 8. Four different quadrants were made in each pot with a clear demarcation. Same number of barley seeds were sown in each quadrants. For monitoring the average shoot and root height, all barley plantlets were removed from each quadrant on each successive day. a. Water only group b. Organic manure and isolate ALKP 12 group. c. ALKP 12 group. d. Positive control group (pseudomonas species).e. Organic manure group f. chemical fertilizer group

2a **2b**

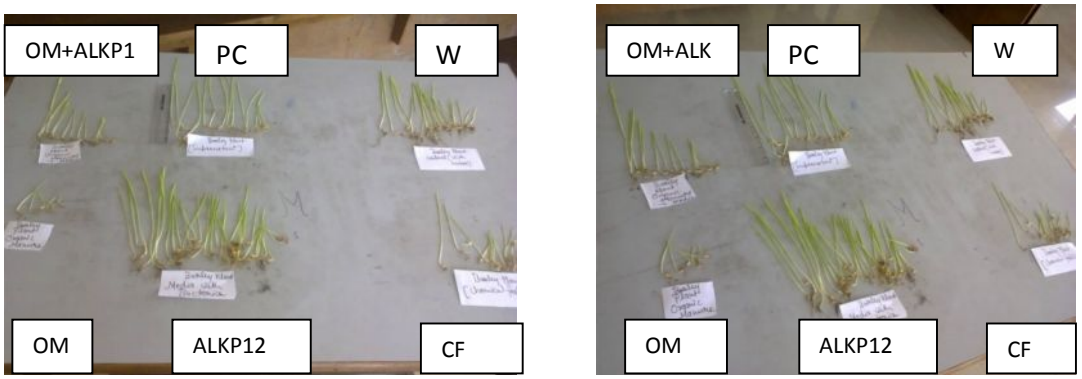


Fig. 2. From the 5th day onwards all the barley plantlets were removed from each quadrants for the analysis of shoot and root height after their various treatments. a.Day 6 plants b.Day 7 plants.

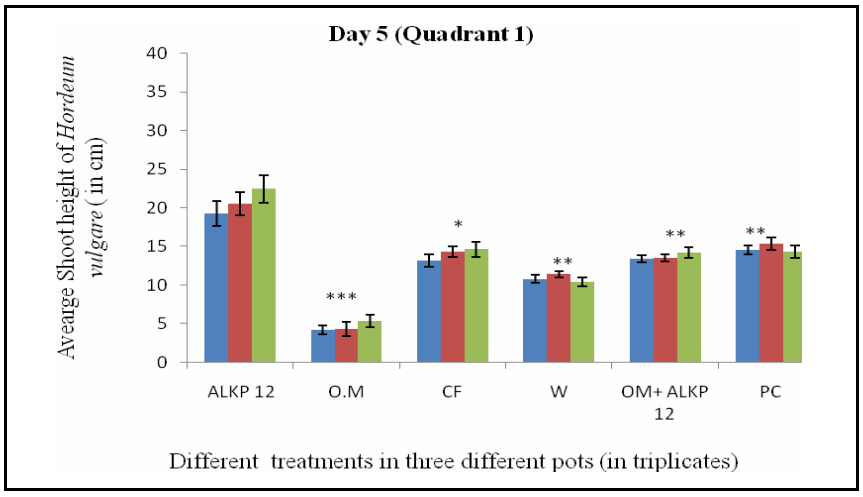
3a **3b**

3c **3d**

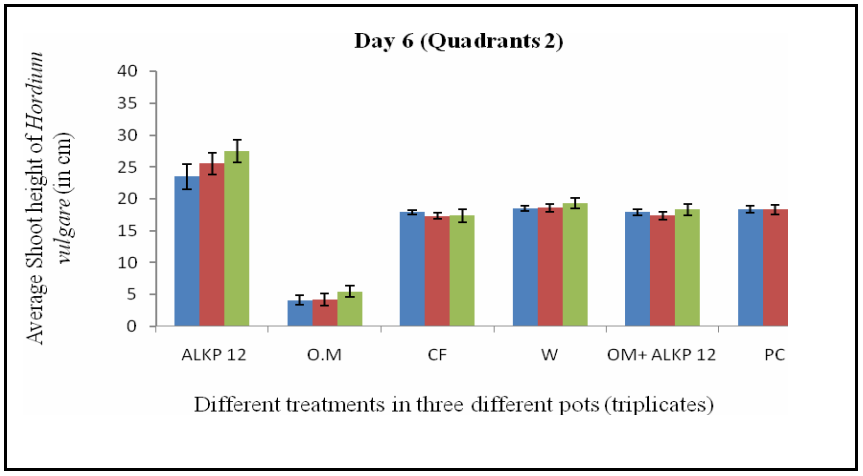
3e **3f**

Fig. 3. T-test and p- values were calculated for all the experiment. These figure shows 6th day shoot and root height with isolate ALKP 12 and was compared with other treatments. From the figure it was clear that isolate ALKP 12 shows a significant increase in the height of both the shoot and root when this type of treatment was compared with other five treatments including positive controls. All p values below are Vs. ALKP12 group a. ALKP 12 group. b. Organic manure alone ($p \leq 0.0006$ for shoot height and $p \leq 0.0001$ for root height) c. Chemical fertilizer ($p \leq 0.013$ for shoot height and $p \leq 0.0019$ for root height) d. Positive control (pseudomonas sp) ($p \leq 0.008$ for shoot height and $p \leq 0.0001$ for root height). e.Organic manure and isolate (ALKP 12 ($p < 0.009$ for shoot height and $p < 0.0004$ for root height) f. Water alone group ($p \leq 0.008$ for shoot height and $p \leq 0.002$for root height)

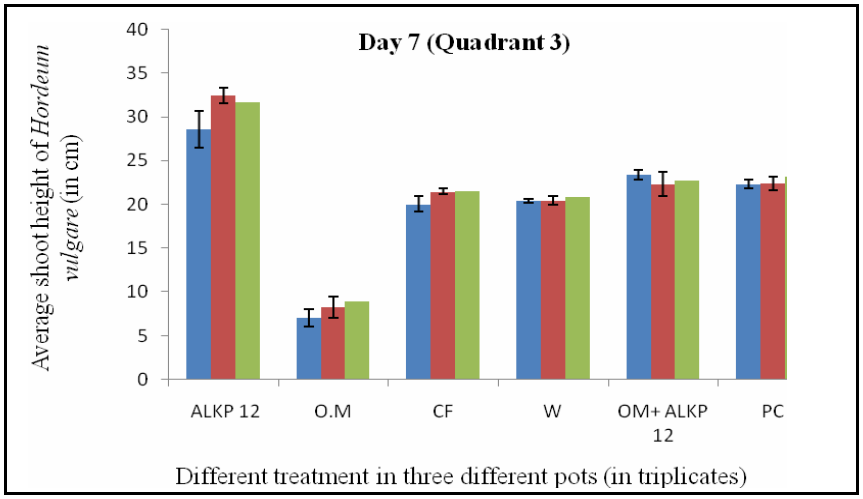
4a


Day 5 (Quadrant 1)
Average Shoot height of Hordeum vulgare (in cm)
40
35
30
25
20
15
10
5
0
***
*
**
**
**
ALKP 12
O.M
CF
W
OM+ ALKP 12
PC
Different treatments in three different pots (in triplicates)

4b


Day 6 (Quadrants 2)
Average Shoot height of Hordium vulgare (in cm)
40
35
30
25
20
15
10
5
0
ALKP 12
O.M
CF
W
OM+ ALKP 12
PC
Different treatments in three different pots (triplicates)

4c


Day 7 (Quadrant 3)
Average shoot height of Hordeum vulgare (in cm)
40
35
30
25
20
15
10
5
0
ALKP 12
O.M
CF
W
OM+ ALKP 12
PC
Different treatment in three different pots (in triplicates)

4d

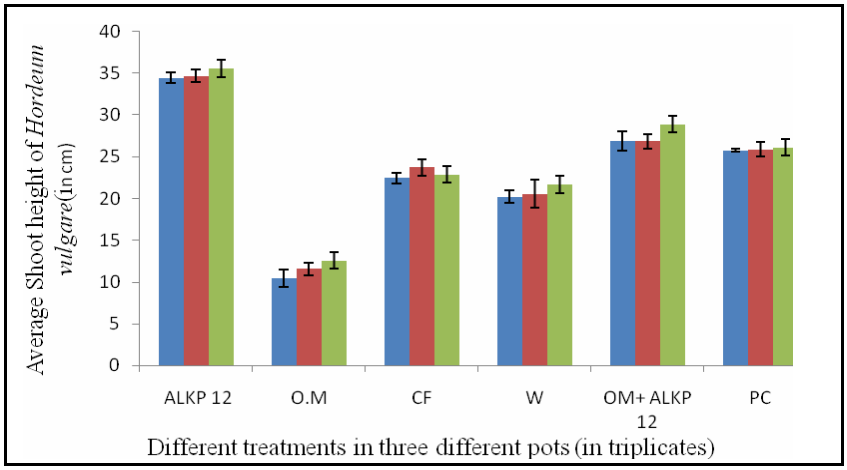
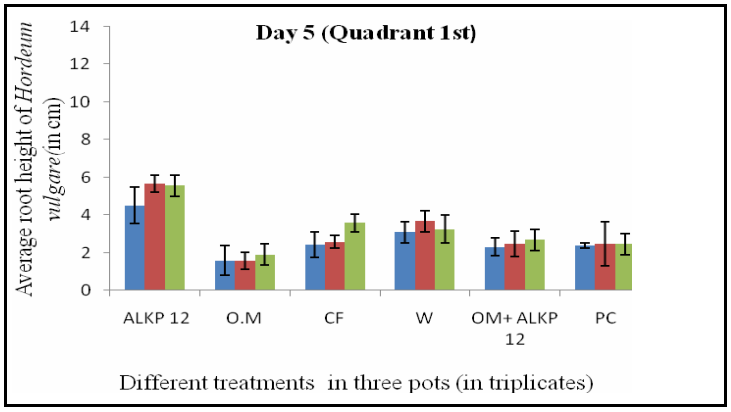
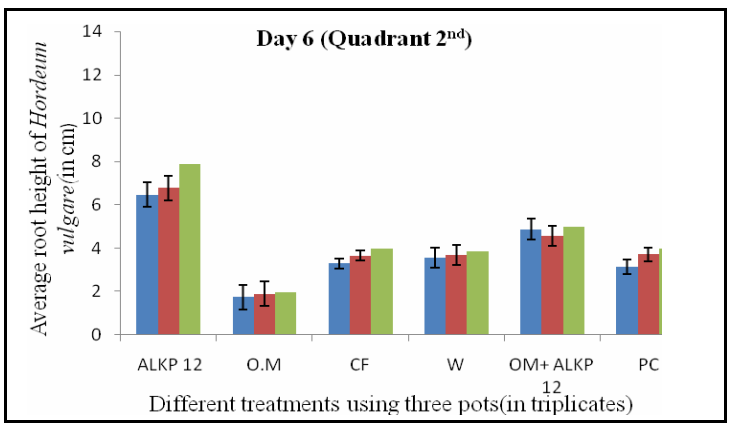


Fig. 4.Observations of six different treatments(in triplicates) of the *Hordeum vulgare*. L seeds. Data on shoot length is represented here. a. Data of day five shows that ALKP 12 alone group has significantly higher length of shoot and root as compared organic manure($p<0.0003$), chemical fertilizer($p<0.001$), water ($p<0.005$), organic manure amalgum ($p<0.004$), positive control($p<=0.003$) b. Data of day six. ALKP 12 Vs. organic manure ($p<0.0006$), chemical fertilizer ($p<0.01$), water ($p<0.008$), organic manure ($p<0.009$), positive control ($p<0.007$). c. Day seven, ALKP 12 Vs. organic manure ($p <0.01$), chemical fertilizer ($p<0.013$), water ($p<0.012$), organic manure +ALKP 12 ($p<0.02$), positive control ($p<0.03$). d. Day eight. ALKP 12 Vs. organic manure ($p< 0.0001$), chemical fertilizer ($p<0.0009$), water ($p<0.0003$), organic manure + ALKP 12 ($p<0.001$), positive ($p<0.002$)

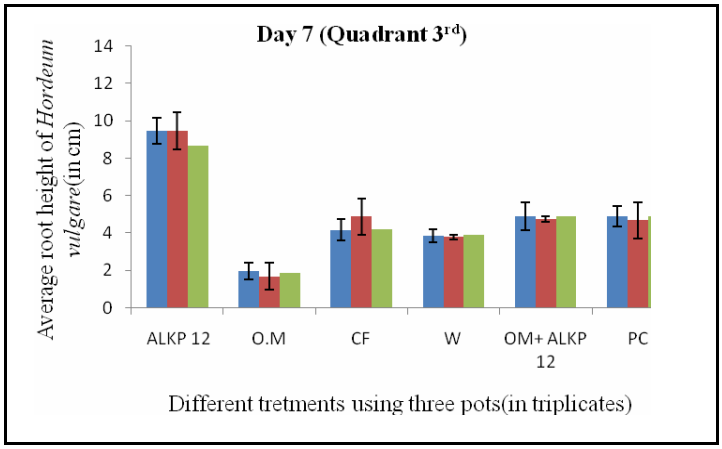
5a



5b



5c



5d

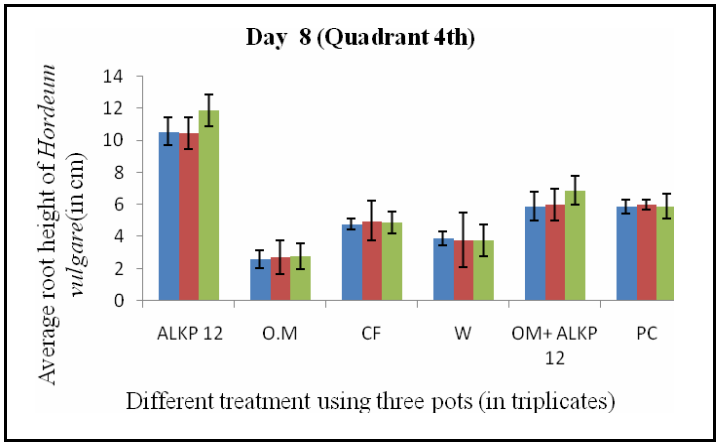


Fig. 5. Effect of different treatment groups (in triplicates) root growth of *Hordeum vulgare*. L. From 5th day onwards till 8th day, all the barley plantlets were removed from pots and the length of roots were measured. a. Data of day five clearly shows that ALKP 12 has best growth of length of root height of *Hordeum vulgare*. ALKP12 Vs. organic manure ($p<0.0003$), ALKP Vs. chemical fertilizer ($p<0.0001$), water ($p<0.0009$), ALKP Vs. organic manure + ALKP 12($p<0.0005$), Vs. positive control ($p<0.0006$) b. Data of day six. ALKP 12 Vs. organic manure ($p<0.0001$), chemical fertilizer ($p<0.002$), water only ($p<0.001$), organic manure ($p<0.0004$), positive control ($p<0.0002$). c. Data of Day seven. There was again a significant increase in the length of root when compared with other treatments d. Similar pattern were observed on Day 8.

Conclusions

When the study of bacterial isolate (ALKP 12) was carried on the seedlings of the model plant (*Hordeum vulgare*) to see the growth pattern on the shoot and root continuously from the day 5 till day 8 done in triplicates even after the experiments repeated several times there was a significant high increase in the shoot length and root length when it was compared with chemical fertilizer, organic manure, water, organic manure + isolate and a positive control. Although our results conclude that the isolate ALKP 12 could be a good source for the manufacture of bio-inoculants/bio-fertilizer but many further investigations are needed especially with actual increase of phosphate levels in soil and plant – microbe interaction. Our present study is only limited to eight days of root and shoot growth of *Hordeum vulgare*. However the measurement of the whole plant growth (both the shoot and root) till the fruiting stage shall have to be carried for further validation along with phosphorus content, change in the soil before and after the treatment are still in the process..

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