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# Response of Mungbean (*Vigna radiata* L. Wilczek) to Rhizobium inoculation and Irrigation Schedule in Siraha, Nepal

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## ABSTRACT

Mungbean (*Vigna radiata* L.) productivity in Nepal is indeed lower compared to its potential yield due to inadequate nutrient management and soil moisture depletion. Use of effective plant growth promoting bacteria and proper irrigation scheduling in mungbean can enhance its production and productivity. To study the response of Rhizobium inoculation and irrigation schedule on growth and yield of mungbean, a field experiment was conducted in a split-plot field layout at a farmers' field in Dhangadimai Municipality, Siraha, Nepal from March 2023 to June 2023. The treatments consisted of four levels of irrigation (i.e. Rainfed, Irrigation at vegetative stage (V), irrigation at reproductive stage (R) and irrigation at both (V and R) in main plots and two levels of biofertilizer (with and without Rhizobium inoculation) in sub plots which was replicated three times. Plant height, leaf numbers, number of effective branches, pod length, and grain per pod were recorded highest in mungbean inoculated with rhizobium, and irrigation at both V and R stages. The highest grain yield (1.81 $Mtha^{-1}$) was obtained from the treatment having irrigation at both V and R stages, followed by irrigation scheduled at R (1.56 $Mtha^{-1}$), V (1.47 $Mtha^{-1}$), and rainfed condition (1.34 $Mtha^{-1}$). The grain yield was higher in inoculated (1.58 $Mtha^{-1}$) than in non-inoculated mungbean (1.50 $Mtha^{-1}$). Rhizobium inoculated crops irrigated during V stage only, irrigated during R stage only and irrigated at both V and R stages increased grain yield by 6.32%, 5% and 17% respectively as compared to rainfed conditions. Number of effective nodules per plant was 79 % higher in inoculated (15.72) than in non-inoculated plots (4). The best combination was found in the treatment where irrigation was applied in both V and R stages with Rhizobium inoculation. Thus, application of rhizobium inoculation and irrigation at V and R stages showed a positive response to growth parameters, yield attributing characters, and yield.

**Keywords**: Irrigation, Mungbean, Productivity, Rhizobium

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## INTRODUCTION

Mungbean is an important pulse crop of Nepal. It is mostly cultivated as a spring crop under rice-wheat-mungbean patterns in irrigated or moderately irrigated areas in the terai, inner terai, and warm valleys. It can be produced as a supplementary crop following the harvest of winter crops such as winter wheat, winter legumes, and oilseeds and before growing main season rice (Neupane et al 2003). As it can provide nitrogen needs through biological nitrogen fixation, it may be beneficial to replace the fallow (Haque and Sattar, 2010; Khanal et al 2004). The production and productivity of mungbean in Nepal is 6500 Mt and 0.5 $Mtha^{-1}$ respectively (MoALD 2020). The main causes for the low mungbean yield during cultivation are inadequate soil moisture and insufficient soil nutrients. It is imperative to schedule irrigation at the most crucial time and regulate nutrients to improve mungbean output.

Pulse crops do not respond favorably to chemical fertilizers to meet their nutrient requirements especially nitrogen, bio-fertilizers being ecofriendly, non-hazardous and non-toxic; play important role to meet nutritional requirements. Among different bio-fertilizers, Rhizobium plays an important role in nitrogen fixation (Sharma 2016). They facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the form of biocontrol agents (Ahemad 2014; Agele et al 2017). Moisture plays a crucial role in the effectiveness and health of Rhizobium bacteria in mungbean cultivation. It enhances Rhizobium bacteria's survival, nodulation, and nitrogen fixation in mungbean, leading to improved plant growth and yield. Water-deficient soil conditions can cause disruption to plant physiology and microorganisms in the soil (Ramadhani et al 2020). Moreover, Drought stress negatively impacts plant establishment, osmotic behavior, photosynthetic capabilities, and metabolic processes consecutively slowing down the development of plants with loss of yield and productivity around the world (Sapna 2021). Therefore, rhizobium and sufficient moisture work together to maximize nitrogen fixation, promote nutrient availability, improve root nodulation, raise plant resistance, and dramatically increase mungbean yields and growth.

Mungbean has the potential to provide multiple benefits to farmers, consumers, and the environment. However, there are still many challenges to overcome to realize the full potential of the crop. Factors like poor soil fertility, lack of nitrogen fixation, moisture stress, the incidence of pests and diseases, production in marginal land, low experience of a farmer, inadequate application of fertilizer, and no knowledge of seed inoculation with nitrogen-fixing bacteria have restricted the growth, development, and yield of Mungbean. Thus, the study is conducted to explore innovative approaches like Rhizobium inoculation and irrigation scheduling to increase crop productivity, and reduce the environmental footprint of farming systems.

## MATERIALS AND METHODS

**Description of Experiment Site:**The experiment was conducted from $10^{th}$ March 2023 to $10^{th}$ June 2023 and located in the terai belt of Nepal at $26^{0}44.76^{'}$ N latitude and $86^{0}22.91^{'}$ E longitude with an elevation of 63.5 meter above sea level. The experiment site had subtropical monsoon climate with distinct summer and winter seasons (Figure 1).

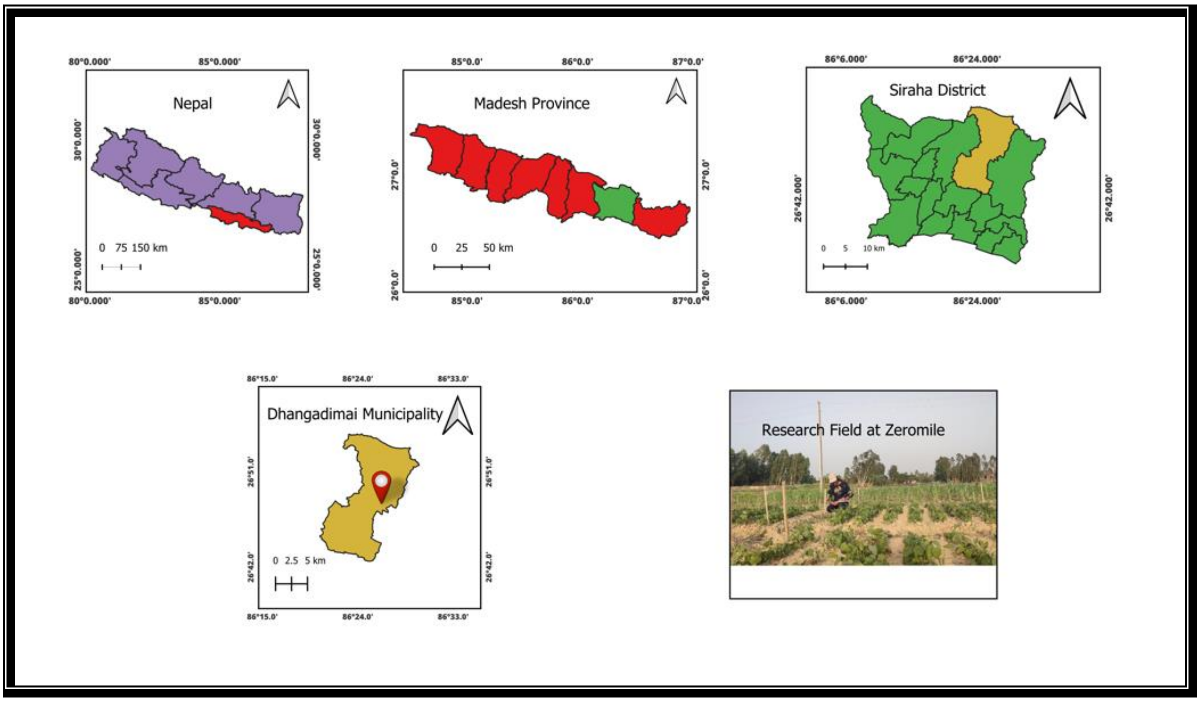


Figure 1. Map of Nepal indicating the experimental site at Zero-mile, Siraha, Nepal

**Physicochemical Properties of Soil of Research Site**

Composite soil samples were randomly taken from different spots from 0-15 cm depth of experimental plots using a soil auger. The collected sample soils were air-dried. Visible roots and organic residues were removed, and portions of the air-dried samples were crushed, sieved (2mm), and analyzed to record the initial soil physio-chemical properties. The soil of experimental soil was silty loam in texture with low pH, low organic matter, total nitrogen, available phosphorus and high potassium.

**Experimental Design**

The experiment was carried out in split-plot design with four replications and eight treatments. The eight treatments were the integration of different schedules of irrigation and rhizobium inoculation where irrigation was the main plot factor and Rhizobium inoculation was the subplot factor. Pratiksha variety was taken as a test crop for the experiment. There were 32 plots each 3.2 m length and 3 m breadth. Plants were spaced at 1m between replication, 1 m spacing between the main plot, and 0.5 m between the sub plot. Each plot consists of an area of 9.6 $m^2$ and a net experimental area of 180 $m^2$ (Figure 2).

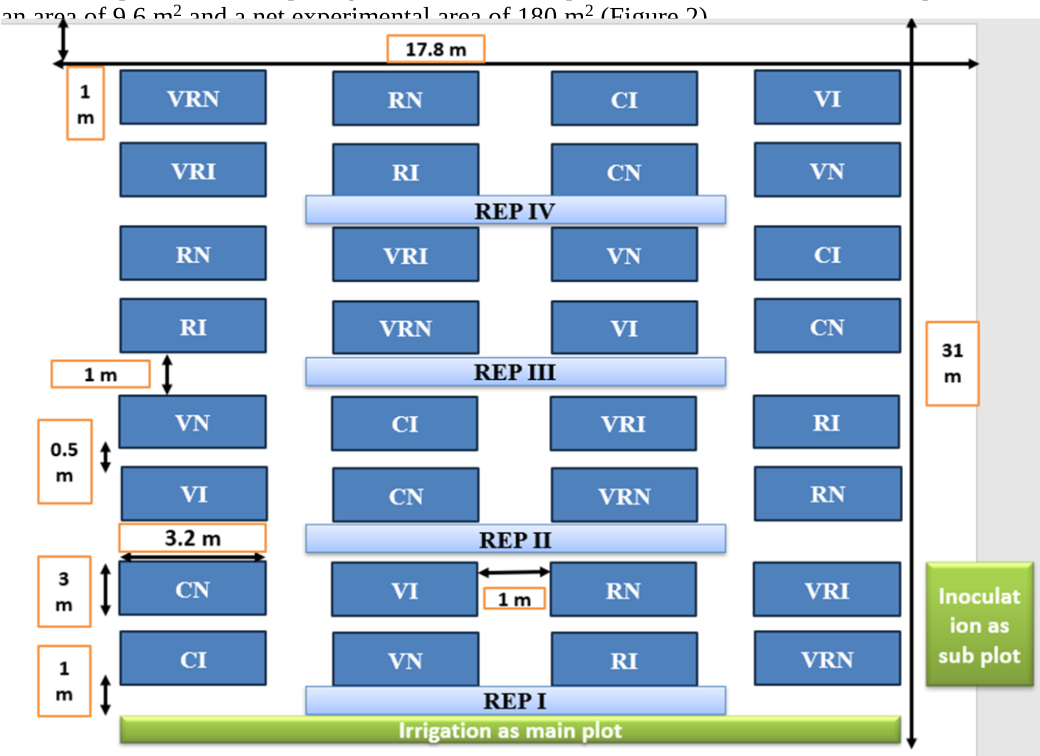


Figure 2. Layout of the experimental design

**Treatment**

Factor 1 (main plot factor): Irrigation schedules

I. Rainfed (Control)
II. Irrigation at the vegetative stage (V)
III. Irrigation at the reproductive stage (R)
IV. Irrigation at both vegetative and reproductive stages (V and R)

Pre-sowing and post-sowing irrigation were given uniformly in each plot. After the plants fully germinated, irrigation was supplied according to the treatment. Irrigation during the vegetative period included irrigating the field until the first flowering, and the reproductive period included irrigating the field until the pods matured

Factor 2 (sub- plot factor): Rhizobium inoculations

I. With Rhizobium inoculation
II. Without Rhizobium inoculation

The seeds were treated with rhizobium bacteria at a rate of 20 gram per kg of seed a day before sowing. 100 grams of jaggery was dissolved in 250 ml of warm water and the seeds were coated and mixed properly. Then, it was spread

**Field preparation, fertilizer, weed and irrigation management**

Primary tillage was done and FYM was incorporated in the field fifteen days before sowing the seeds and harrowing was done one day before sowing. The seed of mungbean was sown on the 25$^{th}$ March 2023 in lines 15cm apart continuously at the rate of 20kg/ha. Nitrogen, phosphorus, and potassium were applied at 20:40:20 kg NPK/ha respectively in each plot in the form of urea, DAP, and MOP. A full dose of fertilizer was used as a basal dose. Weeding was done once manually after 30 days after sowing. Pre-sowing and post-sowing irrigation were given uniformly in each plot and after the full germination of the plant irrigation was supplied according to the treatment where irrigation at the vegetative period included irrigating the field until the first flowering and the reproductive period includes irrigating the field till maturity of the pod.

**Data collection and analysis**

The sample plants under the plot were taken for the measurement of the various characteristics. First data recording was done at 30 days after sowing and regular data recording was done at an interval of 15 days. Phenological traits such as plant height, number of leaves per plant, number of branches per plant, days to 1st flowering, days to 1st pod initiation, number of nodules per plant, number of effective and ineffective nodules, and yield attributing characteristics like number of pods bearing branches per plant, 1000 grain weight, pod length and yield were recorded.

**Harvesting**

The mature pods were picked manually as they ripen. The final harvest was done, when 80% of the pods were ripe. Threshing was manually done by beating the pods with a stick and trampling by feet. Grain was cleaned by winnowing and dried in the sun to reduce the seed moisture content by up to 11%.

**Data Analysis**

The collected data were spread in the MS Excel sheet and subjected to calculate mean, and analysis variance. R-Studio package version R-4.2.2 was used for data analysis. A significant result was separated at 5% and 1% levels of significance by Duncan's multiple range test (DMRT).

## RESULTS AND DISCUSSIONS

**Plant height and number of leaves**

Plant height and number of leaves were significantly influenced by irrigation schedule and inoculation with exception of inoculated plot at 30 DAS (Figure 3). The tallest plant as well as more number of leaves were observed in the treatment where irrigation was applied at both vegetative and reproductive stage followed by irrigation at vegetative stage, reproductive stage and rainfed. Inoculated plot had taller plant height (29.66 cm and 37.12cm) and more number of leaves (8.98 and 11.17) were observed at 45 and 60 DAS respectively. Uddin et al (2013) observed that plant height increased with more number of irrigation, while stressed conditions led to shorter plants, likely due to inhibited cell division and enlargement. Thalooth et al (2006) found that eliminating irrigation during the vegetation, flowering, and pod formation stages of growth significantly reduced all growth parameters, photosynthetic pigments, yield components, and Mungbean yield. Similar results were confirmed by Agele et al (2017) where Rhizobium-inoculated bean seedlings exhibited considerably higher growth characteristics, particularly plant height and leaf number as compared to the non-inoculated species (Figure 3).

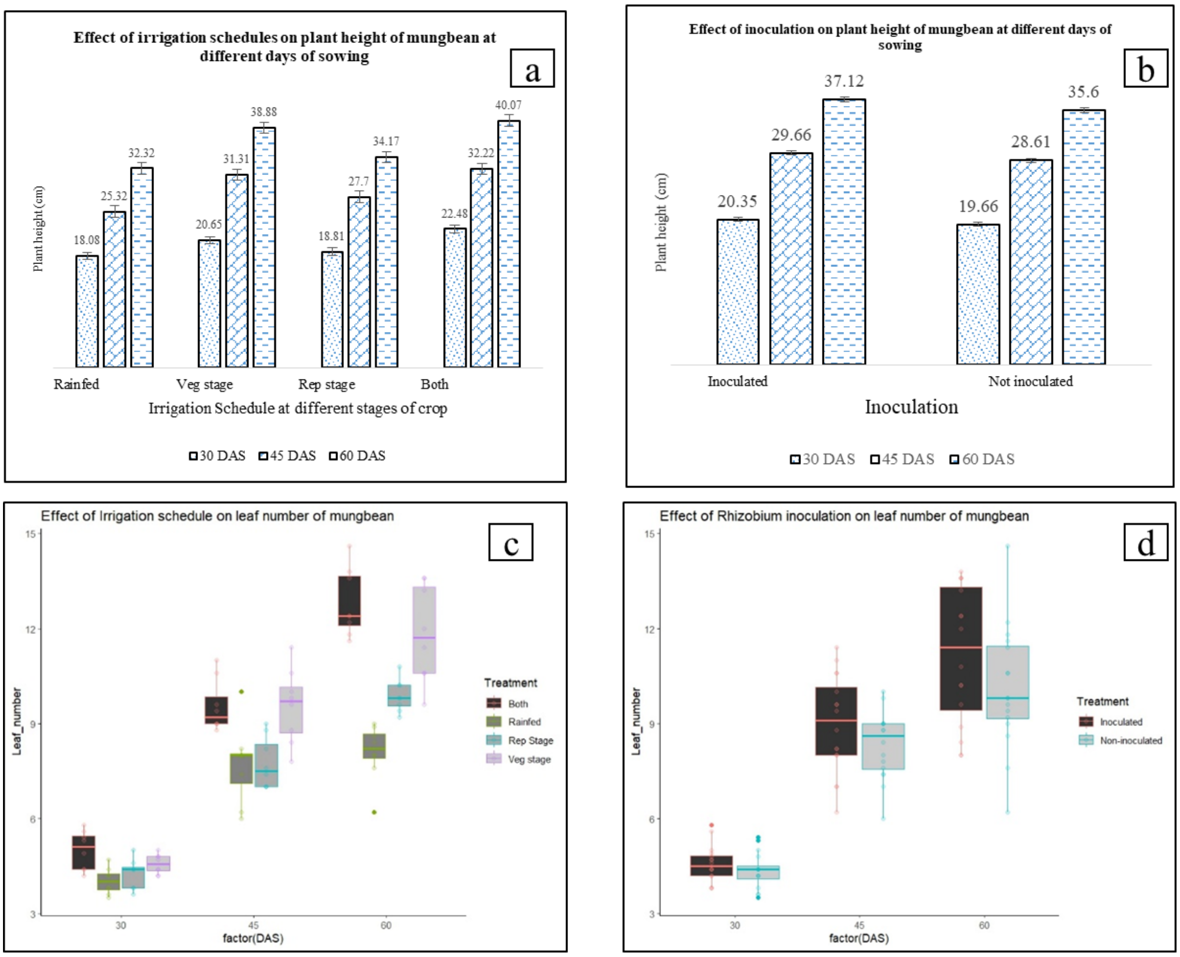


Figure 3. Effect on plant height of mungbean due to irrigation schedule (a) and Rhizobium inoculation (b) at different days of sowing and effect on leaf number of mungbean due to irrigation schedule (c) and Inoculation (d) at different days of sowing

**Number of effective branches per plant, days to first flowering, first pod initiation, no. of nodules and no. of effective nodules per plant.**

The effective branches per plant, number of nodule and effective nodule plant$^{-1}$ were significantly influenced by irrigation schedule and inoculation but in case of days to 1$^{st}$ flowering and 1$^{st}$ pod initiation, they were significantly influenced by irrigation scheduling only but not by rhizobium inoculation (Table 1). Irrigation at both stages had significantly more no of effective branches per plant (3.28), no of nodules (18.44), and no of effective nodule (11.88) followed by irrigation at the reproductive stage, irrigation at the vegetative stage and rainfed plots (1.45), (13.25) and (7.13) respectively. Similarly, inoculated plots had the highest no of effective branches per plant (2.41), no. of nodules (20.85) and no. of effective nodule (15.72) than un-inoculated plots (2.24), (12.38) and (4). A similar finding was observed by Istanbuli et al (2022), who said that drought stress had a negative impact on all nodules observed by inhibiting their growth. This was also supported by Rahman et al (2008) who observed that Seed inoculation with Rhizobium significantly increased nodule number as compared to that of the non-inoculated plants of mungbean.

Significantly more days were required for 1$^{st}$ flowering (39.25) and 1$^{st}$ pod initiation (43.63) in the plot applied with irrigation at the vegetative stage. No significant effect was seen due to rhizobium inoculation. Similar results were observed in Istanbuli et al (2022) which stated that plants cultivated in rainfed environments compared to plants grown under irrigated conditions, flowers blossomed and developed earlier. The results were in accordance with Islam et al (2021) who observed that three irrigations during the growth period of mungbean lengthened the growing seasons.

**Table 1. Number of effective branches (plant$^{-1}$), Days to 1$^{st}$ flowering and days to 1$^{st}$ pod initiation, no. of nodule and effective nodule per plant of Mungbean as influenced by Irrigation schedule and Rhizobium inoculation at Zeromile, Siraha (2023)**

| Treatments | Number of effective branch plant | Days to 1$^{st}$ flowering | Days to 1$^{st}$ pod initiation | No of nodule /plant | No of effective nodule/plant |
|---|---|---|---|---|---|
| **Irrigation** | | | | | |
| Rainfed | 1.45$^{d}$ | 36.00$^{b}$ | 40.13$^{b}$ | 13.25$^{b}$ | 7.13$^{c}$ |
| Veg stage | 2.03$^{c}$ | 39.25$^{a}$ | 43.88$^{a}$ | 18.13$^{a}$ | 10.00$^{b}$ |
| Rep stage | 2.56$^{b}$ | 36.13$^{b}$ | 40.88$^{b}$ | 16.64$^{a}$ | 10.44$^{ab}$ |
| Both | 3.28$^{a}$ | 39.00$^{a}$ | 43.63$^{a}$ | 18.44$^{a}$ | 11.88$^{a}$ |
| SEm (±) | 0.497 | 0.24 | 0.24 | 0.26 | 0.50 |
| LSD (=0.05) | 0.318 | 0.78 | 0.75 | 2.657 | 1.591 |
| CV, % | 12.1 | 1.8 | 1.6 | 14.1 | 14.3 |
| **Inoculation** | | | | | |
| Inoculated | 2.41$^{a}$ | 37.50 | 42.13 | 20.85$^{a}$ | 15.72$^{a}$ |
| Not inoculated | 2.24$^{b}$ | 37.69 | 42.13 | 12.38$^{b}$ | 4.00$^{b}$ |
| SEm (±) | 0.01 | 0.20 | 0.22 | 0.30 | 0.29 |
| LSD (=0.05) | 0.066 | Ns | Ns | 1.632 | 0.917 |
| CV, % | 3.77 | 2.2 | 2.1 | 12.8 | 12.1 |
| Grand mean | 2.33 | 37.59 | 42.13 | 16.61 | 10.02 |

Note: DAS: Days After Sowing, SEm: Standard error of the mean, ns: non-significant, LSD: least significant difference, CV: Coefficient of variation, Treatment means followed by common letter/s within the column are not significantly different from each other based on DMRT 0.05.

**Yield and yield attributing character of mungbean**

The yield and yield attributing character i.e. pod length, no of unfilled pod plant $^{-1}$, grain pod$^{-1}$, and test weight were significantly influenced by irrigation schedule and inoculation as presented in Table 2. Significantly, more grain yield (1.81t ha$^{-1}$), test weight (43.16), pod length (8.83 cm), and grain per pod (11.06) were observed in the treatment where irrigation was applied at both vegetative and reproductive stage and least in the rainfed plot. While more no. of unfilled pod was observed in rainfed plot (5.50) and less in the plot where irrigation was applied at both stage (3.00). These findings were supported by Raza et al (2012) and Islam et al (2021), who found that varied irrigation levels had a significant influence on the number of pods per plant, grain per pod, grain yield, and 1000 seed weight. It was also in accordance with Sadasivan et al (1988) who observed that water stress during the vegetative stage, reduces seed yield due to limited plant size, leaf area, and root growth, thereby reducing dry matter accumulation, fruit number per plant and weak growth ability. Similarly, the research conducted by Uddin et al (2013) found that increased drought at the reproductive organ development stage has a pronounced effect on fruit development and yield. The increase in floral and pod abortion rates was the key factor contributing to the yield loss brought on by drought stress. In case of inoculation, plot with rhizobium inoculation recorded significantly more grain yield (1.58 t ha$^{-1}$), test weight (42.30), pod length (8.68), and grain per pod (10.79). Similarly, Number of unfilled pods was observed less in inoculated plot (3.56) as compared non inoculated plot (4.38). Moreover, significant effect was observed in grain yield of mungbean due to the interaction between irrigation schedule and rhizobium inoculation.

Grain yield of (1.90 t ha$^{-1}$) was recorded as highest in the combination of irrigation at both vegetative and reproductive stage with inoculation and least in the combination of rainfed without inoculation (1.31 t ha$^{-1}$) (Table 2). Bam et al (2022), also observed that rhizobium inoculation enhances root nodulation through greater root growth and increased nutrient availability, resulting in enhanced grain production. A similar result was observed in Bhatnagar (2022) who demonstrated the use of Rhizobium + PSB + might improve yield parameters such as number of pods/plants, pod length, number of seeds/pods, seed yield/plant, and test weight.

**Table 2. Yield and yield attributing character of mung bean as influenced by irrigation schedule and Rhizobium inoculation at Zeromile, Siraha, (2023)**

| Treatments | Yield and Yield attributing character of mung bean | | | | |
|---|---|---|---|---|---|
| | **Pod length (cm)** | **Grain yield (Mt ha$^{-1}$)** | **Number of unfilled pod/plant** | **Grain/pod** | **Test weight (g)** |
| **Irrigation** | | | | | |
| Rainfed | $7.91^{b}$ | $1.34^{d}$ | $5.50^{a}$ | $9.54^{b}$ | $40.60^{c}$ |
| Veg stage | $8.50^{a}$ | $1.47^{c}$ | $4.13^{b}$ | $10.74^{a}$ | $41.60^{b}$ |
| Rep stage | $8.81^{a}$ | $1.56^{\mathbf{b}}$ | $3.25^{c}$ | $10.79^{a}$ | $42.57^{a}$ |
| Both(V+R) | $8.83^{a}$ | $1.81^{a}$ | $3.00^{c}$ | $11.06^{a}$ | $43.16^{a}$ |
| SEm (±) | 0.24 | 0.18 | 2.07 | 0.21 | 0.02 |
| LSD (=0.05) | 0.404 | 0.041 | 0.682 | 0.56 | 0.653 |
| CV, % | 4.2 | 2.4 | 15.2 | 4.7 | 1.4 |
| **Inoculation** | | | | | |
| Inoculated | $8.68^{a}$ | $1.58^{a}$ | $3.56^{b}$ | $10.79^{a}$ | $42.30^{a}$ |
| Not inoculated | $8.34^{b}$ | $1.50^{b}$ | $4.38^{a}$ | $10.27^{b}$ | $41.67^{b}$ |
| SEm (±) | 0.06 | 0.04 | 0.12 | 0.08 | 0.13 |
| LSD (=0.05) | 0.180 | 0.037 | 0.377 | 0.243 | 0.405 |
| CV, % | 2.8 | 3.2 | 12.3 | 3 | 1.3 |
| Grand mean | 8.51 | 1.54 | 3.97 | 10.53 | 41.98 |

Note: DAS: Days After Sowing, SEm: Standard error of the mean, ns: non-significant, LSD: least significant difference, CV: Coefficient of variation, Treatment means followed by common letter/s within the column are not significantly different from each other based on DMRT 0.05.

**Table 3. Grain yield (Mtha$^{-1}$) of Mung bean as influenced by the interaction of irrigation schedule and Rhizobium inoculation at Zeromile, Siraha, (2022)**

| Irrigation scheduling | Inoculation | |
|---|---|---|
| | **With inoculation** | **Without inoculation** |
| Rainfed | $1.362^{f}$ | $1.31^{f}$ |
| Vegetative stage | $1.487^{de}$ | 1.45e |
| Reproductive stage | $1.578^{c}$ | $1.533^{cd}$ |
| Both stage(V+R) | $1.90^{a}$ | $1.72^{b}$ |
| SEm (±) | 0.024 | |
| LSD (=0.05) | 0.075 | |
| CV, % | 2.8 | |
| Grand mean | 1.54 | |

Note: DAS: Days After Sowing, SEm: Standard error of the mean, ns: non-significant, LSD: least significant difference, CV: Coefficient of variation, Treatment means followed by common letter/s within the column are not significantly different from each other based on DMRT 0.05.

## CONCLUSION

Mung beans differed significantly in their response to irrigation schedule and rhizobium inoculation based on the measured parameters. The maximum yield of mung bean was obtained when irrigation was applied at both the vegetative and reproductive stages followed by irrigation at the reproductive stage, irrigation at the vegetative stage, and rainfed. In the case of inoculation, all the growth, as well as yield and yield attributing parameters, was found better in the inoculated plot as compared to the non-inoculated plot. The best combination was found in the treatment where irrigation was applied in both stages with inoculation. Rhizobium inoculation can improve nitrogen fixation in Mung bean root nodules. Mung beans may efficiently use this fixed nitrogen to boost their growth when paired with adequate watering, resulting in healthier plants and perhaps better yields. A sufficient supply of water and better nitrogen availability via Rhizobium can promote flowering, pod development, and seed production, resulting in a greater yield. Irrigation ensures that the plants receive enough water to sustain pod growth, while rhizobium inoculation supplies nitrogen, which is required for pod formation and filling.

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**AUTHOR'S CONTRIBUTION**

P Chaulagain conceptualized original draft and did formal analysis, investigation methodology. SK Sah did visualization, validation, writing-review and editing. A Jaishi did supervision, validation, writing-review and editing. We all read and accept the manuscript.

**CONFLICT OF INTEREST**

The authors declare no competing interests relevant to the content of this article.

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