Original Research Article

Response of 1-Aminocyclopropane-1-carboxylic acid containing *Pseudomonas fluorescens* for rhizobial population and yield of Maize (*Zea mays* L.)

ABSTRACT

Pseudomonas (P) *fluorescens* promote plant growth by lowering the endogenous ethylene synthesis in the roots through their 1-aminocylopropane-1-carboxylate-deaminase (ACCD) activity. However, in the environs of the roots may decrease the efficiency of these *P. fluorescens* by stimulating ACC- oxidase activity resulting in greater ethylene production by the roots. So this study was designed to assess the performance of *P. fluorescens* containing ACC-deaminase for improving rhizobial population and yield of maize. The results revealed that the rhizobial population, irrespective of treatments, was maximum at 55 days after sowing followed by a reduction at harvest. The treatment T_{28}+pH 7.2 (Temperature 28^{O} C + pH 7.2) enhanced the maximum population of *P. fluorescens* in rhizoshpere at 35, 55 and 75 days after sowing (DAS) was with 1.69, 1.59 and 1.87 log fold increase over fertilized un-inoculated FUI [(3.7089 log cfu/g = 5.80 x10^{3} cfu g^{-1} soil), (5.8673 log cfu/g = (7.53x10^{5} cfu g^{-1} soil) and (4.676 log cfu/g 5.55x10^{4} cfu g^{-1} soil)]. Similar treatment T_{28}+pH 7.2 recorded maximum number of cob $plant^{-1}$, cob $length^{-1}$, number of grains $line^{-1}$ and number of grains cob^{-1} at harvest were with T_{28+}pH 7.2 by 30.6, 32.1, 29.7 and 37.7% over the control FUI (2.1, 15.2 cm, 35.0 and 503), respectively. While the treatment T_{31}+pH 7.2 (Temperature 31^{O} C + pH 7.2) enhanced the cob girth and maximum grains line cob^{-1} at harvest were with 26.0 and 31.3% response over FUI (13.3 cm $cob^{-1}$and 13.9), respectively. The treatment T_{28}+pH 7.2 significantly increased the seed and stover yields of maize by 24.9 and 31.6% over the control (2914 and 7133 kg ha^{-1}), respectively.

Keyword: ACC-deaminase; Cob; rhizobial; population; yield; Maize

1. Introduction

Maize (*Zea mays* L.) is the most widely distributed cereal crop of the world. India occupies fifth place in acreage and ranks 10^{th} in production. Maize is one of the most versatile emerging crops showing wider adaptability under varied agro-climatic conditions. Due to its highest yield potential among the cereals it is globally known as the queen of cereals. The largest producer of maize is United States of America contributing about 35% of the total world maize production. In India, the major

maize growing states are Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Punjab, Haryana, Maharashtra, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka, and Jammu and Kashmir, jointly accounting for over 95% of the national maize production [1]. The average productivity in India is 1959 kg ha^{-1} grown in 7.18 million hectares area contributing 14.1 MT of production, and the crop is the third most important food crop after rice and wheat [2]. Maize in India, contributes nearly 9% in the national food basket and more than Rs. 100 billion to the agricultural GDP at current prices. The state of Madhya Pradesh occupies 13% of the total maize area and contributes equally to the total maize production in the country. Nutritionally, maize contains 60 to 68% starch, 1.2 to 5.7% edible oil and 7 to 15% protein [3].

P. fluorescens belongs to Pseudomonadaceae family, is a Gram-negative rod shaped bacterium with visible flagella extending from their cell wall, having a ratio of non-mucoid to mucoid cells approximately 9:1 but a thin layer of material known as peptidoglycan outside their cell membrane. These bacteria can secrete a fluorescent pigment 'pyoverdin' (iron binding siderophore) that exhibits bright color under certain conditions. The bacteria are obligate aerobes but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. Moreover, the bacteria are able to regulate their population density through quorum sensing [4].

ACCD was first discovered in soil microorganism *Pseudomonas* sp. and shown to convert ACC (1-aminocyclopropane-1-carboxylic acid), a precursor of hormone ethylene to ammonia and α-ketobutyrate, both of which are further metabolized by a microorganism [5]. It is a multimeric sulfhydryl enzyme with a monomeric subunit having molecular mass of approximately 35-42 kDa, it utilizes pyridoxal 5-phosphate as an essential co-factor tightly bound to the enzyme @ 1 molecule per mol of an enzyme or 1 mol per trimeric subunit [6], required for enzyme activity. The native size of the enzyme in trimeric form with molecular mass of 110-112 KDa has been reported from *Pseudomonas sp.* The enzyme is localized within the cytoplasm of the microorganism that produces it [7]. In this case, the substrate ACC is exuded by plant tissues [8, 9] and is then taken up by the ACC-deaminase containing microbe [10, 11] investigated the performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize in the presence of nitrogenous fertilizer. All the three strains tested had significant positive effect on cob length and caused up to 18.1% increase in cob length under N-unfertilized conditions. Under N-fertilized conditions, two strains, Q14 and N3, significantly increased (up to 14%) cob length over uninoculated control (N fertilized).

the present investigation was carried out to study the "response of 1-Aminocyclopropane-1-carboxylic acid containing *Pseudomonas fluorescens* for rhizobial population and yield of maize (*Zea mays* L.)"

2. MATERIALS AND METHODS

The present lab or field investigation was carried out in 2 phases as Component- I (during rainy season of 2018): kinetics of population growth of *P. fluorescens* and ACCD activity of the bacterium under different conditions of fermentation; and Component- II (during winter season of 2019): performance of selected isolates of *P. fluorescens* on yield of maize. Studies of component- I and II were performed at the Laboratory of Microbes Research & Production Centre and the Research Farm, Department of Soil Science & Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur India, (latitude of 23° 13 ' N and longitude of 79° 57' E with an altitude of 393 m above mean sea level) in the period of Feb-June, 2019. The field soil was deep black, having pH, 7.48; organic carbon; 0.53%, available N- 233 kg ha-^{1}, available P- 14.9 kg ha-^{1}, available K- 209 kg ha^{-1}, and *P. fluorescens* population being - (1.35×104 cfu g-1 soil). Under fermentation experiment a local but effective strain of *P. fluorescens* was batch cultured for 13 intervals of time (0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hr) with 16 treatments including four temperature regimes (i.e., 25, 28, 31, 34°C), four pH (i.e., 6.7, 7.2, 7.7, 8.2) and their interactions under Randomized block designs (RBD) factorial design with 2 replications. Broth samples were drained out from the fermentator at the scheduled intervals for study on the bacterial population growth and enzyme ACCD activity. Under field study, the 16 isolates derived from the treatments of different temperature and pH with additional 2 controls [Total treatments = 18 (16 isolates + 1 FUI + 1 UFUI)] were tried on maize following RBD design with 3 replications.

2.1 Statistical Analysis

Data were statistically treated by ANOVA, RBD to test the statistical significance of variance among different treatment means as influenced with the application of the treatments on various attributes of maize.

2.2. Climate

The tropic of cancer passes through the middle of the district. It has sub-tropical climate characterized by hot dry summers and cool dry winter. Jabalpur lies in the “Kymore Plateau and Satpura hills” agro- climatic zone of Madhya Pradesh. Seasonal variations prevailing during the growing period plays an important role in the developmental process, which may have great influence on the final yield of the crop. The weekly meteorological data during crop season were obtained from Meteorological Observatory College of Agricultural Engineering, JNKVV, Jabalpur and are depicted through Figure 1. It is obvious from the meteorological data that the weather conditions which prevailed during the crop season were favorable for the growth and development of maize. The mean weekly maximum temperature ranged from 26.2 to 44.4°C whereas; mean weekly minimum temperature ranged between 3.1 to 27.3°C. Relative humidity however, varied from 49.0 to 85.0 % in morning and 17.0 to 47.0% in evening. The mean sunshine hours varied between 7.3 to 10.3 h per day. Total rainfall of 77.3 mm was received during the period.

2.3. Seed inoculation, treatment and sowing

The isolates of *P. fluorescens* were obtained from the laboratory experiment (Component I) and the strain performing best in terms of population growth and ACCD enzyme activity were earmarked and specially selected for the field trial on maize to observe sustainability of the attributes. However, remaining other isolates along with the selected isolates were also included in the field trial. The isolates in broth were used for seed treatment and foliar spray on the maize crop at three growth stages (at knee stage, tasselling and silking stage) to ascertain the population growth attributes and ACCD activity in the rhizosphere and the corresponding impact on the plant growth and yield at crop maturity. Maize seeds in polythene bags were slightly moistened and then treated with carbendazim 2 g kg^{-1} seed. Then the seeds were inoculated individually with the isolates of *P. fluorescens* in the recommended dose 10 ml kg^{-1} of seed and using sterilized gum acacia (2%) as adhesive. The field experiment was carried out at research farm JNKVV Jabalpur during the winter season of 2019. The seeds of maize (cv. JM-216) were sown in the respective plots. Recommended package of practices (Depth of Sowing: 4-6 cm, where maize is generally sown on flat beds. Spacing: 70 x 25cm with the corresponding plant density of 66,666/ha and intercultural operation) was followed to maintain plant population, protection and growth. Three plants from each plot were taken to measure cob $plant^{-1}$, cob girth, grain line cob^{-1}, grains $line^{-1}$, grains cob and cob length $^{-1}$ at harvest. To determine the microbial population in soil, the serial dilution method was followed. study was done adopting serial dilution method.

Plating was done by taking 1 ml of 10^{-7} to 10^{-9} dilutions individually in sterilized and solidified King's B medium in Petri plates. Plating was performed in triplicate for each dilution. The plates were incubated upside down at 28±2^{o}C for 24-72 hr. The colonies with specific growth characteristics (smooth, entire circular, convex, opaque, glistening, yellowish green colonies) of *P. fluorescens* were counted. The KB plates were examined for colony morphology and pigmentation daily up to 3 days for bacterial colonies.

Chart 1: Composition of King's B medium for *P. fluorescens* [12]

Ingredients	Quantity (g/litre)
Peptone (proteose)	= 20.0 g
Glycerol	= 10.0 ml
K_2HPO_4	= 1.5 g
$MgSO_4.7H_2O$	= 1.5 g
Agar-Agar	= 15-18 g
Distilled water	= 1000 ml

3. RESULTS AND DISCUSSION

Results of field experiments revealed that inoculation with selected *P. fluorescens* isolates, containing ACCD activity, under field conditions significantly promoted the growth and yield contributing parameters of maize including cob $plant^{-1}$, cob girth, number of grain lines cob^{-1}, number of grains $line^{-1}$, number of grains cob^{-1}, cob length, yield of maize and rhizobial population. However, the rate of

enhancement varied with isolates obtained from component (I). Inoculation of rhizobacterial isolates in the presence of chemical fertilizer significantly increased yield contributing parameters of maize.

***P. fluorescens* population in rhizospheric soil at 35 DAS, 55 DAS and 75 DAS**

The population of *P. fluorescens* in rhizospheric soil at 35 DAS increased from 3.5534 log cfu/g ($4.00x10^3$ cfu g^{-1} soil) to 6.2502 log cfu/g ($3.74 x10^6$ cfu g^{-1} soil) with the average value of 5.066 log cfu/g ($1.01x10^5$ cfu g^{-1}soil). Among all the treatment combinations T_{28}+pH 7.2 responded maximum by 6.2502 log cfu/g ($3.74x10^6$ cfu g^{-1}soil) which is 1.69 log fold increase over control (3.7089 log cfu/g = $5.80 x10^3$ cfu g^{-1} soil), followed by T_{31}+pH 7.2, T_{25}+pH 7.2 and T_{34}+pH 7.2 by 6.1327 log cfu/g ($2.85x10^6$ cfu g^{-1} soil), 5.9417 log cfu/g ($2.85x10^5$ cfu g^{-1} soil) and 5.7893 log cfu/g ($1.47x10^5$ cfu g^{-1} soil) with the responses of 1.65, 1.60 and 1.56 log fold increase, respectively over FUI (3.7089 log cfu/g 5.80 $x10^3$ cfu g^{-1} soil).

The data on *P. fluoroscens* population in rhizospheric soil at 55 DAS ranged from 4.8060 log cfu/g ($6.43x10^4$ cfu g^{-1} soil) to 9.3687 log cfu/g ($3.03x10^9$ cfu g^{-1} soil) with the mean value of 7.4617 log cfu/g ($3.02x10^7$ cfu g^{-1} soil). A close inspection of all the treatment combinations, revels that the response of T_{28}+pH 7.2 was the best by 9.3468 log cfu/g ($3.03x10^9$ cfu g^{-1} soil) 1.59 log fold over the control of FUI (5.8673 log cfu/g = $7.53x10^5$ cfu g^{-1} soil), followed by T_{31}+pH 7.2, T_{25}+pH 7.2 and T_{34}+pH 7.2 by 9.1151 log cfu/g ($2.06x10^9$ cfu g^{-1} soil), 9.0360 log cfu/g ($2.87x10^9$ cfu g^{-1} soil) and 8.8060 log cfu/g ($1.54x10^8$ cfu g^{-1} soil) with the responses of 1.55, 1.54 and 1.50 log fold, respectively over the control of FUI (5.8673 log cfu/g = $7.53x10^5$ cfu g^{-1} soil). The population of *P. fluoroscens* in rhizospheric soil at 75 DAS ranged from 4.4270 log cfu/g ($2.70x10^4$ cfu g^{-1} soil) to 8.7431 log cfu/g ($3.36x10^8$ cfu g^{-1} soil) with the mean value of 6.6848 log cfu/g. Among all the treatments, the treatment combination of T_{28}+pH 7.2 again responded the best by 8.7431 log cfu/g ($3.36x10^8$ cfu g^{-1} soil) with the responses of 1.87 log fold over FUI (4.676 log cfu/g = $5.55x10^4$ cfu g^{-1} soil), followed by T_{31}+pH 7.2, T_{25}+pH 7.2 and T_{34}+pH 7.2 by 8.3962 log cfu/g ($2.69x10^8$ cfu g^{-1} soil), 8.3902 log cfu/g ($3.79x10^8$ cfu g^{-1} soil) 8.4559 log cfu/g ($3.47x10^8$ cfu g^{-1} soil) with the responses of 1.81, 1.80 and 1.79 log fold, respectively over FUI (4.676 log cfu/g = $5.55x10^4$ cfu g^{-1} soil).

The results of the present investigation have shown that increase in microbial population in the rhizosphere soil was possibly due to the improvement in the more availability of nutrients (especially phosphorus) to the microbes. [13] reported that the plant growth promoting rhizobacteria screening and characterization and their Influence on Maize. Result showed that the significantly increased in microbial population count was significant in all inoculated treatments over the controls. *Azotobacter* population increased upto 70 DAS of crop growth in all the treatments and maximum population was observed with 75% RDN+ Nitrogen fixer *Azotobacter*. Increased may be due to root exudates and soil nutrients in the rhizosphere of maize. The result was in harmony with that found by [14, 15, 16].

Yield Attributes

Table 2 depicts the data on number of cob, cob girth, cob length, number of grain lines cob^{-1}, number of grains line^{-1} and number of grains cob^{-1} inoculated with *P. fluorescens* isolates screened out from different fermentation conditions of temperature and pH.

Cob plant^{-1}

The number of cobs plant^{-1} ranged from 1.8 to 2.7 with the mean value of 2.3. The maximum number of cobs plant^{-1} was 2.7 recorded in the T_{28}+pH 7.2 by 28.5% response and it was found statistically superior over the control FUI (2.1 cob plant^{-1}), it was followed by T_{31}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7, T_{31}+pH 7.7 and T_{25}+pH 7.7 with number of 2.6, 2.5, 2.5, 2.4, 2.4 and 2.4 cobs plant^{-1} along with 23.9, 19.0, 19.0, 14.3, 14.3, 14.3 and 14.3% response, respectively.

Girth of cob

Similarly, the girth of cob varies from 12.2 to 16.8 cm cob^{-1} with the average of 14.8 cm cob^{-1}. Among all the treatments, the treatment combination of T_{31}+pH 7.2 increased the girth of cob by 16.8 cm cob^{-1} with 26.3% response over FUI (13.3 cm cob^{-1}), followed by the T_{28}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7, T_{31}+pH 7.7, T_{25}+pH 7.7, T_{28}+pH 6.7 and T_{31}+pH 6.7 with girth of cob 16.5, 16.4, 16.2, 15.9, 15.8, 15.4, 15.2 and 15.0 cm by 24.1, 23.2, 21.7, 19.3, 18.7, 15.5, 14.1 and 12.2% increment over FUI (13.3 cm cob^{-1}).

Cob length

Data on cob length of maize varied from 14.8 to 20.1 cm cob^{-1} having a mean value of 17.6 cm cob^{-1}. The data revealed that the cob length of maize 20.1 cm cob^{-1} recorded by the application of T_{28}+pH 7.2 with an increment of 32.1% over the control FUI (15.2 cm cob^{-1}), was the highest, followed by with that T_{31}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7, T_{31}+pH 7.7, T_{25}+pH 7.7, T_{28}+pH 6.7 and T_{31}+pH 6.7 with cob length19.9, 19.9, 19.4, 19.3, 18.7, 18.3 and 18.2 cm cob^{-1} by 30.8, 30.4, 27.1, 26.7, 22.9, 20.3 and 19.7% response, respectively. While the lowest value of cob length (14.8) cm cob^{-1} was recorded to UFUI.

Grain line cob^{-1}

The number of grain lines cob^{-1} ranged from 13.0 to 18.3 with the mean value of 16.0. The maximum number of grain lines cob^{-1} was 18.3 recorded with T_{31}+pH 7.2 by 31.6% response and it was found statistically superior over the control FUI (13.9 line cob^{-1}) followed by T_{28}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7, T_{31}+pH 7.7, T_{25}+pH 7.7 and T_{28}+pH 6.7 with 18.2, 18.0, 17.7, 17.6, 17.3, 16.9 and 16.6 line cob^{-1}of 30.9, 28.5, 27.3, 26.6, 24.5, 21.6 and 19.4% response, respectively.

Number of grains per line

Similarly, the number of grains per line varies from 30.6 to 45.4 grains per line with the average of 39.2 grains. Among all the treatments, the treatment combination of T_{28}+pH 7.2 recorded increase in the number of grains per lines by 45.4 grain with 29.7% response over FUI (35.0), followed by T_{31}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7, T_{31}+pH 7.7, T_{25}+pH 7.7, T_{28}+pH 6.7, T_{31}+pH 6.7 and T_{34}+pH 6.7 with the number of grains per line 44.1, 43.9, 43.2, 43.2, 41.7, 41.1, 40.7, 39.8 and 38.5 by 26.0, 25.2, 23.4, 23.3, 19.1, 17.4, 16.2, 13.6 and 9.8% increment over FUI (35.0 grains line^{-1}).

Grains cob^{-1}

A close inspection on the data on number of grains cob^{-1} of maize varied from 483 to 693 grains having a mean value of 604 grains cob^{-1}. The data revealed that the highest number of grains cob^{-1} of maize (693) was recorded by the application of T_{28}+pH7.2 with an increment of 37.7% over the control FUI (503 grains cob^{-1}) followed by T_{31}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7, T_{31}+pH 7.7, T_{25}+pH 7.7, T_{28}+pH 6.7, T_{31}+pH 6.7, T_{34}+pH 6.7, T_{25}+pH 6.7 and T_{28}+pH 8.2 with grains cob^{-1} 681, 673, 665, 658, 645, 643, 637, 625, 618, 600 and 582 by 35.3, 33.8, 32.1, 30.7, 28.1, 27.8, 26.8, 24.1, 22.8, 19.2 and 15.7% response, respectively.

In addition to ACC-deaminase potential, these selected isolates also exhibited varied potential of multifarious properties including auxin production, siderophore production, phosphorous solubilization, chitinase activity and root colonization. A similar result was also observed by [17] results showed that inoculation with selected strains of bacteria, exhibited percent increase in yield of fresh cob (up to 52.69%) and dry cob (40.87%), cob length (51.42%), grain yield (up to 55.34%), 1000-grain weight (up to 37.27%) compared to un-inoculated control. [18] showed that the effect of *Azotobacter* on growth and yield of maize. Results showed that showed that grain yield, plant height, ear height, ear length, kernel per rows and 1000 grain weight were significantly affected with *Azotobacter* inoculation over control.

Grain and Stover yield

The data related to the grain and stover yield of the maize crop are given in Table 3. The grain yield of maize ranged from 2440 to 3641 kg ha^{-1} with the average value of 3189 kg ha^{-1}. Among all the treatments, the treatment combination of T_{28}+pH 7.2 yielded significantly maximum grain yield of 3641kg ha^{-1} that was responded 24.9% over FUI (2914 kg ha^{-1}). The stover yield of maize increased from 5874 to 9387 kg ha^{-1} with the mean value of 7934 kg ha^{-1}. The highest stover yield of maize 9387 kg ha^{-1} was recorded with the treatment combination of T_{28}+pH 7.2 by 31.6% responses over the control FUI (7133 kg ha^{-1}). Improvements in growth and yield following application of rhizobacterial isolates under fertilized conditions could primarily be attributed to their ACC-deaminase trait which might have reduced endogenous biosynthesis of C_2H_4 in developing roots of seedlings, and hence resulted in formation of

healthy and longer roots for efficient nutrient uptake. Indigenous C_2H_4 biosynthesis is accelerated during seed germination which has been known for inhibitory effects on seed germination and root growth. In addition to the positive attributes of ACC-deaminase containing PGPR application enhanced nutrient uptake, growth and yield of maize is most likely due to promotion of root growth by the decreased ethylene levels attributed to ACC-deaminase activity [11]. [19] reported that the growth promotion and yield enhancement of barley cultivars using ACC-deaminase producing *P. fluorescens* strains. Results indicated that inoculation with PGPRs had positive impact on growth parameters and yield of barley cultivars including plant height, spike length, weight and number, peduncle length, number of grains per spike, 1000-grain weight and grain yield, comparing to uninoculated control plants under salinity stress. Similarly, improvement in growth indices and yields-of different crop plants like rice, maize and wheat in response to inoculation with PGPR were reported earlier by [20, 21].

5. Conclusion

On the basis of findings, it may be concluded that the maize inoculation with ACCD containing *P. fluorescens* isolates. The use of these effective isolates of *P. fluorescens* could be an effective way to enhance the microbial population and yield attributes of maize.

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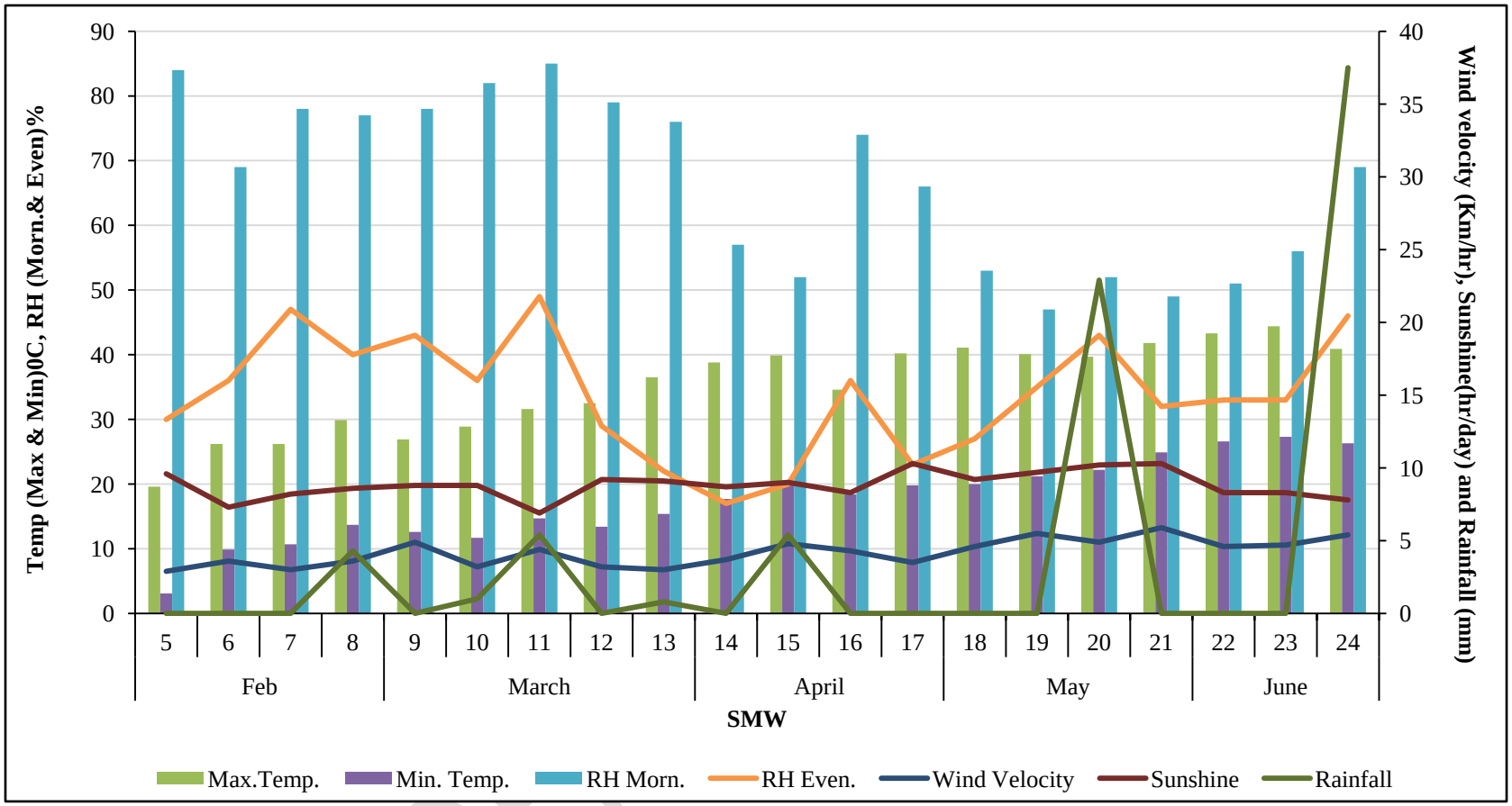

Fig. 1: Weekly meteorological data during Feb - Jun, 2018-19

Table 1. Effect of *P. fluorescens* isolates on rhizospheric population of maize at different growth stages

Treatment combination	***P. fluorescens* population (cfu g^{-1} soil)**		
	35 DAS	55 DAS	75 DAS
T_{25}+pH 6.7	5.0162 ($2.82x10^5$)	7.1177 ($3.55x10^7$)	6.3292 ($2.97x10^6$)
T_{25}+pH 7.2	5.9417 ($2.85x10^5$)	9.0360 ($2.87x10^9$)	8.3902 ($3.79x10^8$)
T_{25}+pH 7.7	5.5157 ($8.85x10^5$)	8.0306 ($1.26x10^8$)	7.7947 ($5.56x10^7$)
T_{25}+pH 8.2	4.2077 ($2.19x10^4$)	6.3751 ($2.52x10^6$)	5.7262 ($5.45x10^5$)
T_{28}+pH 6.7	5.4473 ($4.59x10^5$)	7.9531 ($3.21x10^7$)	6.5486 ($3.47x10^6$)
T_{28}+pH 7.2	6.2502 ($3.74x10^6$)	9.3468 ($3.03x10^9$)	8.7431 ($3.36x10^8$)
T_{28}+pH 7.7	5.7103 ($3.50x10^5$)	8.3886 ($4.61x10^8$)	7.6702 ($1.13x10^7$)
T_{28}+pH 8.2	4.9043 ($2.16x10^4$)	6.7271 ($1.13x10^6$)	6.1408 ($2.58x10^6$)
T_{31}+pH 6.7	5.3373 ($3.92x10^5$)	7.8320 ($2.57x10^7$)	6.2086 ($2.52x10^6$)
T_{31}+pH 7.2	6.1327 ($2.85x10^6$)	9.1151 ($2.06x10^9$)	8.3962 ($2.69x10^8$)
T_{31}+pH 7.7	5.6207 ($6.05x10^5$)	8.2027 ($2.26x10^8$)	7.6062 ($5.52x10^7$)
T_{31}+pH 8.2	4.6618 ($2.42x10^4$)	6.5447 ($2.93x10^6$)	5.7141 ($1.08x10^5$)
T_{34}+pH 6.7	5.2136 ($2.09x10^5$)	7.5609 ($5.32x10^7$)	6.4896 ($3.30x10^6$)
T_{34}+pH 7.2	5.7893 ($1.47x10^5$)	8.8057 ($1.54x10^8$)	8.4559 ($3.47x10^8$)
T_{34}+pH 7.7	4.1156 ($1.94x10^4$)	6.3535 ($4.09x10^6$)	5.6477 ($4.35x10^5$)
T_{34}+pH 8.2	4.0686 ($2.25x10^4$)	6.2471 ($2.16x10^6$)	5.3599 ($2.71x10^4$)
FUI (Control)	3.7089 ($5.80x10^3$)	5.8673 ($7.53x10^5$)	4.6763 ($5.55x10^4$)
UFUI (un-fertilized un-inoculated)	3.5534 ($4.00x10^3$)	4.8060 ($6.43x10^4$)	4.4270 ($2.70x10^4$)
$SE_m\pm$	0.4	0.6	0.2
$CD_{5\%}$	1.3	1.7	0.7

Table 2. Effect of *P. fluorescens* isolates on cob filling of maize

Treatment combination	Cob plant^{-1}	Cob girth (cm)	Cob length (cm)	Grain lines cob^{-1}	Number of grain line^{-1}	Number of grain cob^{-1}
T_{25}+pH 6.7	2.3	14.2	17.1	15.5	38.2	600
T_{25}+pH 7.2	2.5	16.4	19.9	18.0	43.9	674
T_{25}+pH 7.7	2.4	15.4	18.3	16.9	41.1	643
T_{25}+pH 8.2	2.1	14.5	16.3	14.9	35.8	547
T_{28}+pH 6.7	2.3	15.2	18.2	16.6	40.7	637
T_{28}+pH 7.2	2.7	16.5	20.1	18.2	45.4	693
T_{28}+pH 7.7	2.4	15.9	19.3	17.6	43.2	658
T_{28}+pH 8.2	2.2	14.0	16.7	15.2	37.7	583
T_{31}+pH 6.7	2.3	15.0	17.8	15.8	39.8	625
T_{31}+pH 7.2	2.6	16.8	19.9	18.3	44.1	681
T_{31}+pH 7.7	2.4	15.8	18.7	17.3	41.7	645
T_{31}+pH 8.2	2.1	13.7	16.6	15.1	36.2	573
T_{34}+pH 6.7	2.3	14.5	17.2	15.7	38.5	618
T_{34}+pH 7.2	2.5	16.2	19.4	17.7	43.2	665
T_{34}+pH 7.7	2.1	13.5	16.0	14.4	35.2	537
T_{34}+pH 8.2	2.1	13.4	15.8	14.2	35.2	514
FUI (Control)	2.1	13.3	15.2	13.9	35.0	503
UFUI	1.8	12.2	14.8	13.0	30.6	483
$SE_m\pm$	0.07	0.5	1.0	0.8	1.1	24.8
$CD_{5\%}$	0.2	1.4	2.8	2.4	3.3	73.2

Table 3. Effect of *P. fluorescens* isolates on grain and stover yield of maize

Treatment combination	Yield (kg ha^{-1})	
	Grain	Stover
T_{25}+pH 6.7	3033	7502
T_{25}+pH 7.2	3552	9205
T_{25}+pH 7.7	3353	8257
T_{25}+pH 8.2	2950	7247
T_{28}+pH 6.7	3319	8037
T_{28}+pH 7.2	3641	9387
T_{28}+pH 7.7	3452	8900
T_{28}+pH 8.2	2999	7485
T_{31}+pH 6.7	3235	7855
T_{31}+pH 7.2	3590	9248
T_{31}+pH 7.7	3427	8305
T_{31}+pH 8.2	2977	7357
T_{34}+pH 6.7	3182	7756
T_{34}+pH 7.2	3478	8964
T_{34}+pH 7.7	2918	7150
T_{34}+pH 8.2	2935	7140
FUI (Control)	2914	7133
UFUI	2440	5874
SE_m±	142.5	233.9
$CD_{5\%}$	420.0	689.3