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Induction of Antioxidants by PGPR to Mitigate Stress by Pathogen *F. oxysporum* in Chickpea Variety KWR-108 under Drought

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ABSTRACT: The present study was conducted during 2019-2020 and 2020-2021 at MBGE Department, RTMNU, Nagpur, Maharashtra to assess the effects of PGPR with biocontrol activity on antioxidant parameters under drought conditions in situ (field) studies. Three potential PGPRs namely *Pseudomonas* spp, *E. cloacae* and *P. chlororaphis* were used in the present study. The pooled data of two experiment results of antioxidant enzymes recorded T7 (A+B) PGPR treatment with consortium of *Pseudomonas* spp. and *P. chlororaphis* ranged highest in proline with 5.37 (µg/g). In SOD highest recorded with 8.13 (U/mg) in T7 (A+B), PAL ranged highest between 1.76 (mg/L), catalase with 15.56 (µg/g) in T7 treatment according to the pooled data. However the lipid peroxidation and H_2O_2 content indicated as toxic levels caused by over production of ROS during water deficit stress. This could overcome by decreasing levels of MDA through PGPR T7 treatment with 8.67 (Mm^{-1} cm^{-1}), while increased in T3 with 12.39 (Mm^{-1} cm^{-1}) thus improving cellular metabolism and decreasing the disruption of membrane lipids. H_2O_2 content decreased in T7 with 2.05%, while increased in T7 with 7.73% indicating toxicity levels. Therefore according to the results of the current reports, T7 (A+B) with consortium of *Pseudomonas* spp. and *P. chlororaphis* found to be best PGPR treatment with comparing to other treatments in both experiments against *Fusarium oxysporum* and for mitigating drought stress in KWR-108 variety of chickpea.

Keywords: Chickpea, *Fusarium oxysporum*, PGPR, drought, Biochemical.

INTRODUCTION

It ranks 5th among grain crops and is imperative due to its high nutritive contribution in Indian diet. It has considerable importance in fodder also (Montenegro *et al.*, 2010). Chickpea is a vital protein source with nutritional values and high energy (Hulse *et al.*, 1994). It is rich in dietary fibre, zinc, different minerals and B-group vitamins. The sugar, carbohydrate and fat contents is comparatively higher in chickpea than other pulses (Jukanti *et al.*, 2012).

Fusarium wilt caused by FOC *Fusarium oxysporum ciceri* is vital destructive vascular diseases of chickpea. They produce toxins, fumonisins and trichothecenes. The limiting factor in chickpea production majorly including India reported worldwide is Fusarium wilt defined by Butler in 1918, and further, determined its etiology in 1940 by Padwick **(**McKerral, 1923), as it is considered as soil borne fungal disease which belongs to the *Fusarium* genus and therefore reduces both seed weight and seed yield in chickpea production**.**

In spite of the huge reports reported on enzymatic antioxidant activities and their role in regulating abiotic stresses, the caspase activity acts as a key regulator of abiotic stress responses are limited and appears this activity initiation through abiotic stresses, possibly transduced by production of ROS and hence crisis like cold, drought stresses are vital in chickpea production (Singh *et al.*, 1994).

PGPR exhibiting antagonistic effects fungal pathogens which are soil borne in the entire lifespan of plant and stimulates the systemic resistance. Oxidative damage caused by the imbalance in electron transport rates (ETR) (Rahdari *et al.*, 2012) and excessive production of reactive oxygen Species (ROS) through the metabolic consumer activity of reductive power which are scavenged by antioxidants enzymes that aids in

regulating of peroxidase, superoxide dismutase (SOD), catalase activated by rhizobacteria, whereas the dagamed caused to proteins, nucleic acids leads to lipid peroxidation production (Islam *et al.,* 2015).

Antioxidants induction by PGPR strain raises the tolerance and might provide helpful tool for declining abiotic stress in plants of wheat and other crops (Sagar *et al.,* 2021). Some of the representative PGPR belong to generation *Azotobacter, Serratia, Micrococcus, Azospirillum, Burkholderia, Pseudomonas, Enterobacter, Bacillus, Erwinia, Flavobacterium* etc (Singh, 2015). The aim of the current study is to assess the PGPR effects with biocontrol activity on antioxidant parameters under drought conditions in situ (field) studies

MATERIALS AND METHODS

The current study of two trails were carried out during 2019-2020 and 2020-2021 at research fields of MBGE Department, RTMNU, Nagpur, Maharashtra.

Chickpea Variety: Chickpea variety KWR 108 was released in the year 1996 by Chandrashekar Azad University of Agriculture & Technology (CSAUAT), Kanpur and seeds were obtained from ICAR-IIPR, Kanpur. The silent features are: seeds are dark brown and small and rresistant to wilt, 130-135 days to maturity and production 20-23Quintal/Hectare.

PGPRs Details: Three PGPRs were obtain culture collection of Department of Biological Science SHUATS Prayagraj (U.P.).

Pathogen Details: Pathogen- *Fusarium oxysporum* obtain from MBGE Department, RTMNU, Nagpur, Maharashtra

Seed treatment: Seeds were priorly soaked overnight by three PGPRs with single and consortium treatments according to compatibility test results.

Treatment details:

T1- control
T2- control (pathogen-*Fusarium oxysporum*)
T3- control (with *drought*)
T4- Isolate A *(Pseudomonas* spp.*)*
T5- Isolate B *(P. chloraphis)*
T6- Isolate C *(E. cloacea)*
T7- Isolate A+B
T8- Isolate A+C
T9- Isolate B+C
T10- Isolate A+B+C

Pot and Field trials: Experiments were carried out during 2019-2020 (Polyhouse conditions) and 2020-2021 at research fields according to RBD layout, MBGE Department, RTMNU, Nagpur, Maharashtra.

Proline determination: 0.5gm of fresh leaf sample was collected from every different treatment and crushed with 3% sulphosalicylic acid of 10ml in mortar and pestle, then filtered by using whatman's No. 1 filter paper. From this filtrate the supernatant was collected in other tubes to avoid mixture, into this 2ml of ninhydrin acid and glacial acetic acid of 2ml was mixed. This reacted kept aside in hot water bath at 90°C for one whole hour. This reaction get completed by placing this test tubes in ice cubes box, then to the reaction mixture, 4 ml was added. The chromophore of toluene has been separated and observed the spectrophotometer readings at 520nm by using and calculated as per the following formula and standard curve (Bates *et al.,* 1973). It was calculated by the formula,

[(µg proline/ml X ml toluene)/115.5µg/µmole]/ [(g sample)/5] = µmoles proline/g of fresh weight.

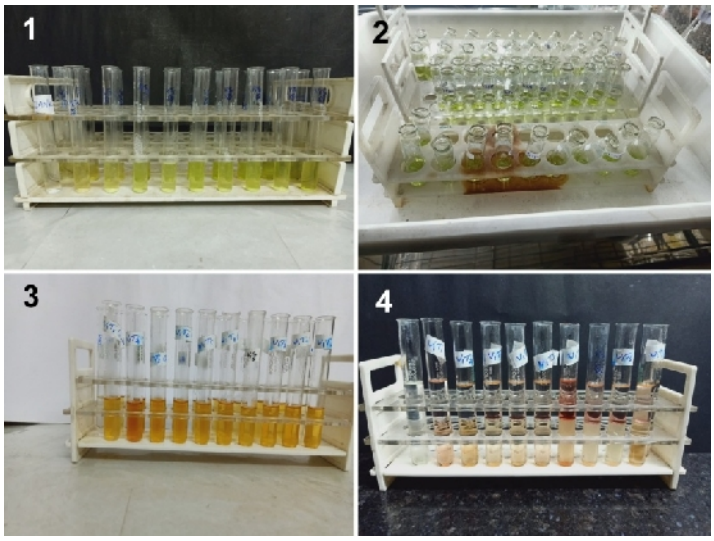
Super oxidase dismutase (SOD): The SOD activity was determined according to the method of (Giannopolitis and Ries, 1977). Sample was collected from the upper most fourth leaf and weighed 0.5g. The plant leaves homogenized with 5ml phosphate buffer solution. 0.5 ml enzyme extract and 0.5 ml distilled water, 2 ml of reaction mixture (75 ml phosphate buffer + 10 ml methionine + 5 ml EDTA + 5 ml NBT + 5 ml sodium carbonate) was added in two tubes one for control tube and second for sample tube. 1ml distilled water and 2ml reaction mixture added in the third tube as a blank tube. At the last 0.1 ml riboflavin added in all tubes and then the reaction was started. Control the blank tubes had shaken, placed in dark and sample tubes were placed under the fluorescent lamp for 30min. Both samples (light and dark) were read the absorbance after 30 minutes in spectrophotometer at 560 nm wavelength and the activity super oxidase dismutase (SOD) was expressed as U mg^{1} protein.

Catalase enzyme activity: 1gm of fresh leaf sample was collected from every treatment separately and crushed with liquid nitrogen 0.1M phosphate buffer (pH 7) with 5ml. This whole mixture was centrifuged for 5 minutes at 10000rpm. To the supernatant 900 µL of distilled water was added, 1.5 ml of 0.1 M phosphate buffer, along with 500 µL of hydrogen peroxide (0.3 %) into the new test tubes. Spectrophotometer reading was at 240 nm (Gopalachari 1963).

Lipid peroxidation: 0.5g of leaf samples collected were crushed with 0.1% TCA upto 10ml make up in a mortar and pestle. Centrifugation was done for 17minutes at 15000rpm. Supernatant has collected about 1ml, to this mixture 0.5% TBA was added and analysed in hot water bath for half an hour at 80° C, repeated this process once more and absorbance has determined of 155 mM^{-1} cm^{-1} (Heath *et al.,* 1968).

Hydrogen Peroxide (H_2O_2): it was estimated through titanium hydro-peroxide formation. To the 5ml of supernatant extract titanium 4ml has been added for whole night to leave for precipitation at 4°C. This was later dissolved by 1M H_2SO_4 of 5ml addition and absorbance was took at 410 nm. With the help of standard curve plots the calculations have been made (Mukherji and Chaudhari, 1983).

Phenylalanine Ammonia Lyase Assay (PAL): 0.5gm of leaf sample collected and crushed with tris buffer 50mM of pH8.8 and centrifuged for 15miniutes at 12000g at 4°C. The mixture consisted of 70µl of 10m M phenylalanine, 176µl of 70mM and 100µl of supernatant extract and allowed to proceed for an hour at 30°C maintaince and terminated the reaction by adding 2N HCl of 200µl. Finally centrifuged repeated the procedure for a while, the collected upper case of supernatant was estimated at spectrophotometer 290nm, as cinnamic acid was used for this observation (Ramamoorthy *et al.*, 2002).



Estimation of antioxidants (1) H_2O_2, (2) SOD, (3) Lipid peroxidation (4) Proline.

Statistical Analysis. The statistical analysed for the data obtained in this current investigation was by two way factor by OPSTAT software RBD (randomized block design). It was corelated to analysis of variance at 5% level and was found to be significant. The pooled was done analyses as per (Gomez and Gomez, 1984).

RESULTS

The further investigation was done under *in situ* conditions in single and consortium treatments of above said three PGPR isolates according to compatibility test results.

A. Proline

During first trail (2019-2020) and second trail (2020-2021) data of both cropping years, T7 (A+B) with PGPR isolates combination (*Pseudomas* spp. and *P. chlororaphis)* recorded proline as highest with 2.6 µg/g and 8.1 µg/g shown in Fig. 1, whereas the pooled data observed maximum in T7 (A+B) with 5.37 µg/g in comparison to other single and consortium PGPR treatments. However the least proline is observed in T3 (treated with pathogen alone) with 0.27 µg/g and 1.29 µg/g during both consecutive years and pooled data with 0.78 µg/g recorded as least in chickpea KWR-108 variety against *F. oxysporum* under limited irrigation conditions.

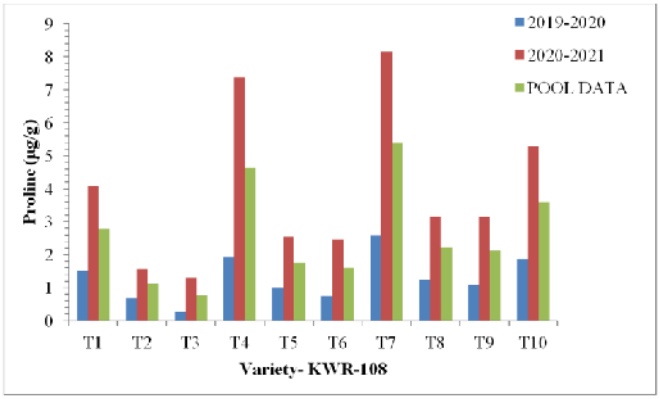


Fig. 1. Graphical representation of variation in proline (µg/g) in chickpea KWR-108 variety due to PGPRs with potential activity against *F. oxysporum* under limited irrigation conditions within two consecutive growing seasons (2019-20 and 2020-21) and pool data.

SOD (super oxide dismutase): During first trail (2019-2020) and second trail (2020-2021) data of both cropping years, T7 (A+B) with PGPR isolates combination (*Pseudomas* spp. and *P. chlororaphis)* recorded SOD as highest with 1.26 U/mg and 1.5 U/mg shown in Fig. 2, whereas the pooled data observed maximum in T7 (A+B) with 8.13 U/mg in comparison to other single and consortium PGPR treatments. However the least SOD is observed in T3 (treated with pathogen alone) with 0.94 U/mg and 2.24 U/mg during both consecutive years and pooled data with 1.59 U/mg recorded as least in chickpea KWR-108 variety against *F. oxysporum* under limited irrigation conditions.

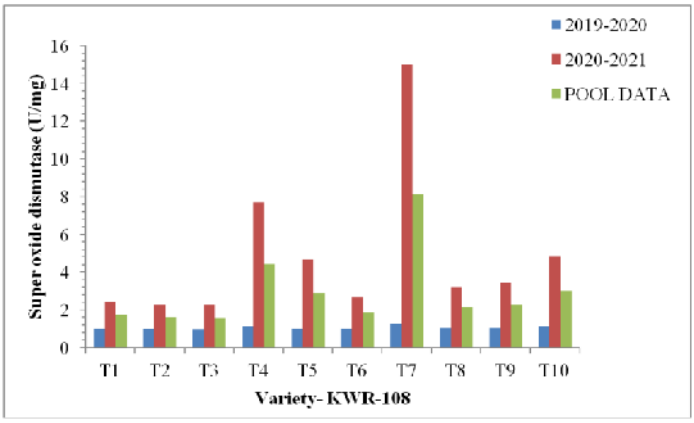


Fig. 2. Graphical representation of variation in SOD (U/mg) in chickpea KWR-108 variety due to PGPRs with potential activity against *F. oxysporum* under limited irrigation conditions within two consecutive growing seasons (2019-20 and 2020-21) and pool data.

PAL (Phenyl ammonia lyase): During first trail (2019-2020) and second trail (2020-2021) data of both cropping years, T10 (A+B+C) with PGPR isolates combination (*Pseudomas* spp. + *P. chlororaphis* + *E. Cloacae)* recorded PAL as highest with 1.82 mg/L and 2.1 mg/L shown in Fig. 3, whereas the pooled data observed maximum in T7 (A+B) with combination (*Pseudomas* spp. and *P. chlororaphis)* as 1.76 mg/L in comparison to other single and consortium PGPR treatments. However the least PAL is observed in T3 (treated with pathogen alone) with 0.55 mg/L and 0.69 mg/L during both consecutive years and pooled data with 0.62 mg/L recorded as least in chickpea KWR-108 variety against *F. oxysporum* under limited irrigation conditions.

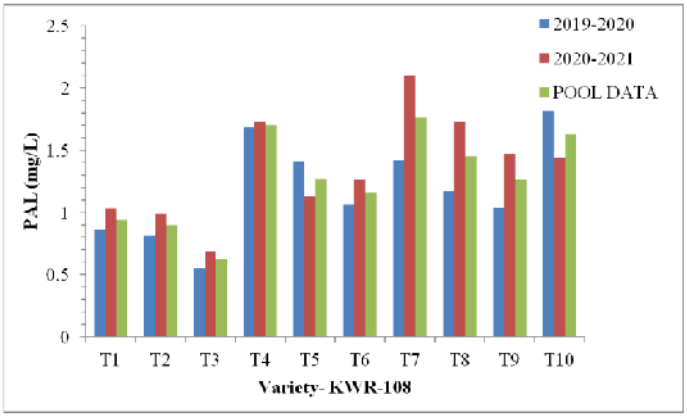


Fig. 3. Graphical representation of variation in PAL (mg/L) in chickpea KWR-108 variety due to PGPRs with potential activity against *F. oxysporum* under limited irrigation conditions within two consecutive growing seasons (2019-20 and 2020-21) and pool data.

Catalase: During first trail (2019-2020) and second trail (2020-2021) data of both cropping years, T7 (A+B) with PGPR isolates combination (*Pseudomas* spp. + *P. chlororaphis)* recorded catalase highest with 1.02 µg/g and 30.1 µg/g shown in Fig. 4, whereas the pooled data observed maximum in T7 (A+B) with 15.56 µg/gin comparison to other single and consortium PGPR treatments. However the least catalase is observed in T3 (treated with pathogen alone) with 0.21 µg/g and 1.98 µg/g during both consecutive years and pooled data with 1.10 µg/g recorded as least in chickpea KWR-108 variety against *F. oxysporum* under limited irrigation conditions.

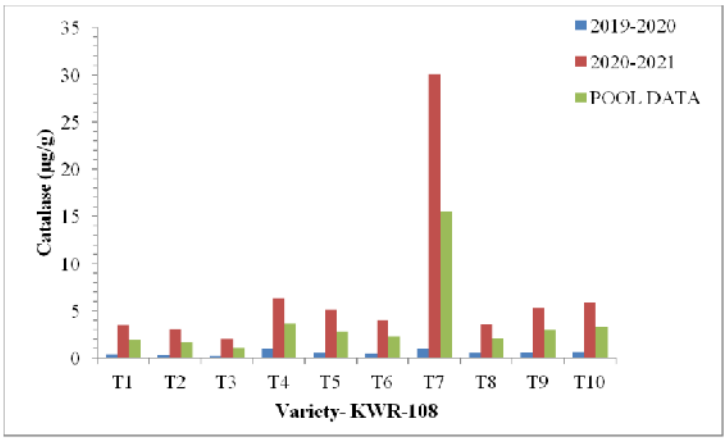


Fig. 4. Graphical representation of variation in catalase (µg/g) in chickpea KWR-108 variety due to PGPRs with potential activity against *F. oxysporum* under limited irrigation conditions within two consecutive growing seasons (2019-20 and 2020-21) and pool data.

Lipid peroxidation: During first trail (2019-2020) and second trail (2020-2021) data of both cropping years, T3 (treated with pathogen alone) recorded lipid peroxidation highest with 8.54 $Mm^{-1}cm^{-1}$ and 16.24 $Mm^{-1}cm^{-1}$ shown in Fig. 5, whereas the pooled data observed maximum in T3 with 12.39 $Mm^{-1}cm^{-1}$ in comparison to other single and consortium PGPR treatments. However, during first trail (2019-2020) the least lipid peroxidation is observed in T4 (A) *(Pseudomas* spp.) single treatment with 0.93 $Mm^{-1}cm^{-1}$ and during second trail (2020-2021) T7 (A+B) consortium (*Pseudomas* spp. + *P. chlororaphis)* with 8.67 $Mm^{-1}cm^{-1}$, while pooled data as 6.27 $Mm^{-1}cm^{-1}$ recorded least indicating ROS scavenging in chickpea KWR-108 variety against *F. oxysporum* under limited irrigation conditions.

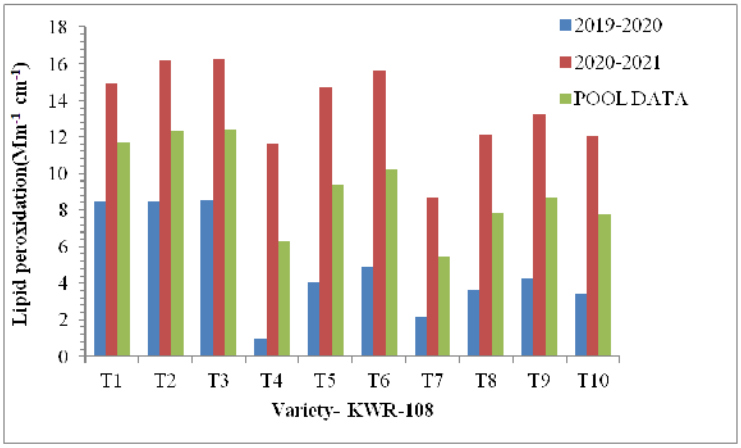


Fig. 5. Graphical representation of variation in lipid peroxidation (Mm^{-1} cm^{-1}) in chickpea KWR-108 variety due to PGPRs with potential activity against *F. oxysporum* under limited irrigation conditions within two consecutive growing seasons (2019-20 and 2020-21) and pool data.

H_2O_2 (Hydrogen peroxide): During first trail (2019-2020) and second trail (2020-2021) data of both cropping years, T3 (treated with pathogen alone) recorded H_2O_2 highest with 4.01 % and 11.44 % shown in Fig. 6, whereas the pooled data observed maximum in T3 with 7.73 % in comparison to other single and consortium PGPR treatments. However, during first trail (2019-2020) the least H_2O_2 is observed in T4 (A) *(Pseudomas* spp.) single treatment with 0.85 % and during second trail (2020-2021) T10 (A+B+C) consortium (*Pseudomas* spp. + *P. chlororaphis* + *E. Cloacae)* with 2.87 %, while pooled data in T7 (A+B) with 2.05 % recorded least indicating ROS scavenging in chickpea KWR-108 variety against *F. oxysporum* under limited irrigation conditions.

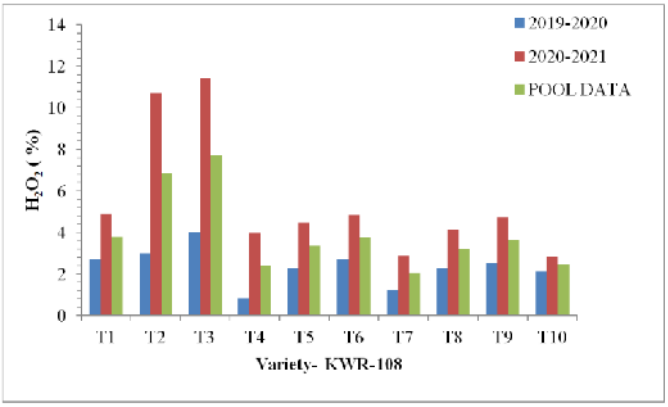


Fig. 6. Graphical representation of variation in H_2O_2 (%) in chickpea KWR-108 variety due to PGPRs with potential activity against *F. oxysporum* under limited irrigation conditions within two consecutive growing seasons (2019-20 and 2020-21) and pool data.

DISCUSSION

Under drought, rhizobacteria are prone to increase compaitable solute concentrations endogenously like proline, since it is most reported osmolyte for its accumulation as well as better indication of tolerating capability of *Pseudomonas* spp. (Sandhya *et al.*, 2010b). This acts as the defender mechanism which provides proteins stability performance and protects from membrane damage under reported pathogen in this study (García *et al.*, 2017).

ROS mainly such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2), hydroxyl (OH^-) and superoxide (O_2-) are formed due to the drought stress conditions, that leads to harsh harm to cell and membrane structures through exertion of cell membrane oxidation well known as oxidative stress (Apel and Hirt, 2004). Conversely, the antioxidant enzymatic system acts as a defensive system which is stimulated by the PGPR stain inoculation of *Pseudomonas* spp. shown in Fig. 1. This contains SOD like various ROS-scavenging enzymes (Mittler, 2002).

PAL transforms L-phenylalanine into trans-cinnamic acid and ammonia, that assists in providing systematic and physiological support through the PGPR treatment with *Pseudomonas* spp has displayed the aggregation of polyphenols in leaves and increase the PAL activity, thus helps to overcome the water stress conditions (Bhattacharyya *et al.*, 2020). PGPR leading host plants inorder to evade pathogen attacks against *Fusarium* spp. in this study through the PGPR defense associated antioxidant enzymatic molecules and production (Choudhary *et al.*, 2007). The various biocontrol mechanisms which helps in systemic resistance as defensive process and also as cell wall degradation through PGPR secreteing enzymes (Hayat *et al.*, 2012).

CAT activity of high production related to H_2O_2, this activity increases during *Pseudomonas* spp inoculated plants with increment of SOD enzyme activity allied to tolerance of oxidative stress through Halli Well Asada pathway (Gong *et al.*, 2005). This helps in conversion of H_2O_2 into O_2, as antioxidative process in ROS. Various literatures found to increase CAT enzyme activity even under drought against reported pathogen on this study (Zhao and Zhang, 2006).

During drought conditions, the activities of MDA were significantly increased indicating toxic levels of cell membrane damage. But the treatment with PGPR consortium of *Pseudomonas* spp and *Enterobacter* spp. the suppressive effect was more dominant and appeared increment activity of above said antioxidants, as the detail may be attributed that PGPR shown in Fig. 1, suppress ROS production and consequently lower MDA content even against *Fusarium* spp. (Jha and Subramanian, 2013).

The ubiquitous life span of H_2O_2 in the atmosphere is dependent on metal ions which are transition state, where some natural spp. can catalyse its decomposition and as well as depends on pH, due to the H_2O_2 presence in water with gas-phase. Catalase, an enzyme which helps in decomposition of H_2O_2 to O_2 in order to eliminate H_2O_2 toxic effects through PGPR inoculation with *Enterobacter* spp and *Pseudomonas* spp. against *Fusarium* spp. even under water deficit conditions (Shaked *et al.*, 2010).

CONCLUSION

From the results, it is concluded that T7 (A+B) is the best combination treatment comprising of *Pseudomonas* spp. and *Pseudomonas chlororaphis* in control of *Fusarium oxysporum* and mitigating drought stress in KWR-108 variety of chickpea in terms of antioxidant parameters such as proline, superoxide dismutase, catalase enzymes increased inorder to overcome negative effects of ROS, whereas lipid peroxidation and hydrogen peroxide decreased due to reported PGPR in this study by decreasing the toxic levels that occur during drought conditions. Therefore PGPR would be suitable reason to conquer reported pathogen even under water deficit conditions.

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Conflicts of interest. None.

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