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Influence of Marigold Flower Effluent and Plant Growth Promoting Microorganisms on Growth and Yield of Potato (*Solanum tuberosum* L.)

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Abstract

Marigold flower leachate, an effluent from the marigold processing industry, was used in irrigation as a one-time amendment to nearby arable land. It has found to be a source of essential nutrients and no harmful effects for plants. Hence, the present study was taken up to evaluate the combined effect of untreated marigold flower leachate (UMFE) and plant growth promoting microorganisms (PGPM) as a supplement along with other combinations, on growth and yield of potato. The treatments T_6 (RDF + UMFE + PGPM) and T_7 (RDF + 50 : 50 (Water : UMFE) + PGPM) reported higher plant height, number of shoots per plant, number of leaves per plant and chlorophyll content and were superior when compared to other treatments. Higher yields were obtained in the treatment received with PGPM, demonstrating that PGPM is effective in stimulating plant vegetative growth and enhancing crop productivity. The yield in T_7 treatment was higher (19.68 t ha^{-1} of tubers), when compared to the control (5.90 t ha^{-1}). The study found that applying UMFE in a 50:50 ratio (UFME: bore well water) to the agricultural land and PGPM, could be a feasible option for recycling the industrial wastewater without losing the essential nutrients. According to the study, UMFE, rich in nutrients improves the soil by adding organic matter, and beneficial bacteria aid in the solubilization and mobilization of nutrients in the soil to the plant system, resulting in a better supplement for improved growth and yield in potato.

Keywords : Plant growth promoting microorganisms, Untreated marigold flower leachate, Potato yield

A number of Indian states suffer from water scarcity, which has become severe in recent years due to population growth, industrialization, global climate change and long lasting periods of drought. Recently, the reuse of waste water in agriculture has become a wide spread practice in water scarce regions. Wastewater reuse not only provides significant amount of irrigation water, but also contributes to conserve potable resources and reduce the environmental impact related to the wastewater discharge into water bodies (Pedrero *et al*., 2010). Further more, soil application of treated waste water also constitutes a reliable source of nutrients (especially nitrogen, phosphorus and potassium) and organic matter useful for maintaining the fertility and the productivity of the soil (Meli *et al*., 2002 and Rusan *et al*., 2015) and also enhance the economic benefits for farmers, due to reduced need for chemical fertilizer Bedbabis *et al*., 2010 and Paranychianakis *et al*., 2006).

Use of agro industries waste water for crop production is gaining importance due to water scarcityfor irrigation and to reduce the ill effects of chemical fertilizers on environment. In the state of Karnataka there are many agricultural produce processing Agro industries *viz*., Cashew processing, Resin processing, Gherkin processing, Wine processing etc., which release huge amounts of effluents after processing, which can be used for irrigation as it has organic source of nutrients. Alike industry is marigold-processing industry located at Hassan, Karnataka for the extraction of oleoresin, xanthophyll and lutein. As marigold flowers contain about 90 per cent moisture, they are dried and used for the extraction of carotenoids that is a coloring agent and used as a nutraceutical in food and pharmaceutical industry by further processing. During the entire process of pressing and dewatering about 40 per cent water drains out and it is collected in a storage tank. It is estimated that about 10,000 - 15,000 tonnes of marigold is processed per year from such industries and approximately about 4 to 6 lakh litres of water comes out every year. This drained marigold flower leachate is a good source of nutrients and can be used as organic liquid fertilizer for crop production as mentioned by Umashankar *et al.* (2019). However, there is a need to evaluate the efficiency of marigold flower leachate on widely grown crops in Karnataka and especially in the district of Hassan.

Potato (*Solanum tuberosum* L.) popularly known as 'The king of vegetable's is grown in more than 100 countries in the world. Karnataka is one of the important potato growing states in the country grown mainly in the districts of Hassan, Belgaum, Chikkaballapur and Kolar. Hassan district alone account for more than 41 per cent potato production in the state (Bhajantri, 2011). In India, it is grown in an area of 2141.72 thousand hectares with the production of 51310.01 thousand tonnes and the productivity is 23.96 MT ha^{-1}. In Karnataka, potato is grown in an area of 35.53 thousand hectares, with an annual production of 509.48 thousand tonnes and productivity accounting for 14.34 MT ha^{-1} (India Agristat, 2017-18).

In correspondence with the reuse of marigold flower leachate from marigold industry, its potentiality can also be assessed in combination with Plant Growth Promoting Microbe (PGPMs). As PGPMs, provide benefits to plants by improving plant nutrient acquisition, inducing plant resistance, pathogen resistance as well as abiotic resistance through influencing key plant hormone pathways, taking advantage of the natural crosstalk existing between these stress-response pathways (Fujita *et al.*, 2006). A study was conducted by Umashankar *et al.* (2011) to evaluate PGPMs on growth promoting activities of Cardamom in nursery, the growth was enhanced in cardamom seedlings, treated with PGPMs. Similar results were obtained in various other crops (Umashankar *et al.,* 2011 and Umashankar *et al.,* 2012).

In view of above facts and in line with the previous research conducted by Umashankar *et al*., 2019, the present study focuses to use the marigold flower leachate as a nutrient and water source along with some PGPMs on growth and yield of potato as it is one of the major vegetable crops grown in Karnataka.

Material and Methods

The experiment was conducted at College of Agriculture, Hassan, University of Agricultural Sciences, Bangalore during *rabi* 2020. The experimental site is geographically situated in the Southern Transition Zone (Zone - 7) of Karnataka and located between 12° 13' and 13° 33' N Latitude and 75° 33' and 76° 38' E Longitude at an altitude of 827 m above Mean Sea Level (MSL). The initial soil properties of experimental site was analyzed using standard methods as described by Jackson (1973).

Table 1

Treatment details

T_1	Absolute Control
T_2	RDF (Control)
T_3	RDF + Plant Growth Promoting Microorganisms (PGPM)
T_4	RDF + Jeevamrutha
T_5	RDF + Untreated Marigold Flower Leachate (UMFE)
T_6	RDF + UMFE + PGPM
T_7	RDF + 50: 50 (Water: UMFE)+ PGPM

RDF : Recommended dosage of fertilizer

The experiment was layed out in Randomised block design with seven treatments and replicated thrice (Table 1). The gross plot size of experimental plot was 3.6 m x 3 m and net plot size was 2.4 x 2.6. Certified seed tubers (cv. *Kufri Jyothi*) of stage II obtained from National Seed Corporation, Jalandar, Punjab were used for planting which were procured from regulated seed market of Hassan. The whole suberized tubers were cut into two or three pieces and were treated with Mancozeb (2 gL^{-1}) for 20 minutes and dried in shade overnight and planted with 60 x 20 cm to prevent the decay of seed tubers.

Recommended dose of fertilizer application of 125 : 100 : 125 kg NPK ha^{-1} was applied in the form of urea, di-ammonium phosphate and muriate of potash, respectively as per the treatments to each plot and mixed well into the soil. Fifty per cent of the recommended nitrogen was applied at the time of planting and remaining fifty per cent was applied four weeks after planting as top dressing. Common irrigation was given to all the treatments during the dry spell as protective irrigation using portable sprinkler.

Before imposing the treatments the water holding capacity of the field was determined by using Keens cup method. The water holding capacity was found to be 12 per cent (250 lts. / plot to attain the field capacity). For the preparation of Plant growth promoting microbes (PGPM) consortia, aloopful of pure cultures (N-fixer + P-solubilizer + *Pseudomonas fluorescens*) were inoculated into 400 mL nutrient broth and incubated at 25 °C for five days. The culture broth was applied along with farm yard manure (FYM) before sowing.

The treatments were imposed 15 days before sowing by flooding the plots with calculated quantities of untreated marigold flower leachate (UMFE). After fifteen days, tuberswere planted in rows by opening furrows with spacing of 60 x 20 cm. All the agronomical practices were carried out as per the recommendation. The growth parameters like plant height, Number of shoots per plant, Number of leaves / plant, chlorophyll content (SPAD units) and yield parameters like Number of tubers/ plant, tuber yield/ hectare (kg ha^{-1}) were recorded by using standard methods of five plants that were randomly selected in each treatment at the time of harvest. Experimental data obtained were subjected to statistical analysis adopting Fisher's method of analysis of variance as out lined by Gomez and Gomez (1984). Obtained data were analyzed for descriptive statistics (mean and standard error of mean) at 5 per cent probability level, where the mean differences were adjusted with Duncan's Multiple Range Test (DMRT) and standard deviation has been mentioned.

Results and Discussion

Experiment was conducted, to know the effect of marigold flower leachate generated by Omni Active Pvt. Ltd. during the processing of Marigold flowers on the growth of potato in field condition and the results are as follows.

Initial soil properties were analyzed before imposing treatments in the field to understand the changes happened in the soil by the irrigation of untreated marigold flower effluent and on application of plant growth promoting micro organisms to enhance the crop productivity. The details of initial soil properties are presented in Table 2.

TABLE 2

Initial soil and microbial properties

Soil properties	Values
pH	8.01
EC (dS m^{-1})	0.20
Available nitrogen (kg ha^{-1})	188.47
Available phosphorous (kg ha^{-1})	38.13
Available potassium (kg ha^{-1})	220.14
Bacterial population (CFU/g of soil)	7.8×10^8
Fungal population (CFU/g of soil)	5.12×10^3
Actinomycetes population (CFU/g of soil)	11.56×10^2

The plant height of potatoes differed significantly due to different treatments at all growth stages. The height of the potato plants at 30 Days After Sowing (DAS) was lower in the plots where only water was given for irrigation and it was 22.16 cm (Table 3). Significantly higher plant height was observed in the plots irrigated with water and untreated marigold flower leachate (UMFE) in the ratio of 50 : 50 along with PGPM (52.89 cm) and it is on par with the treatment T_6 and T_5. The same trend was observed at flowering stage and also at harvest, at flowering the higher plant height was in T_7 treatment (58.89 cm) when compared to PGPM (51.39 cm) and Jeevamrutha (50.83 cm) applied plots and the lower plant height was observed in absolute control plots (28.07 cm). At harvest, T_7 treatment reported maximum plant height of 64.44 cm and was lower with T_1 (33.43 cm). The effluents of marigold industry is a complex mixture of various essential nutrients (Umashankar *et al.*, 2019) that promote plant growth. Some reports revealed that industrial waste water is harmful for crops while others advocated its beneficial aspects. For example, Hussain *et al.* (2010) revealed that tannery effluents lowered sunflower germination and growth, as well as having a negative impact on chlorophyll, protein and carbohydrate content. Singh *et al.* (2011), on the other hand, found that tannery effluents at lower concentrations or after diluting the effluent increased seedling development in Chrysanthemum. According to Pandey *et al.* (2007), the effect of waste water irrigation varies from crop to crop because each plant has its own tolerance limits to different pollutants present in waste water. The findings coincide with the results obtained by Dawwam *et al.* (2013), in their study; they have inoculated *Pseudomonas* isolates to soil and observed an increase in shoot length of sweet potato. These reports substantiate the findings of the current investigation in which UMFE and PGPM were used in tandem.

Similar trend was observed in the number of shoots per plant at 30 DAS it was significantly higher in T_6 treatment RDF + UMFE + PGPM (4.37) and the lower was in T_1. At flowering stage, the same trend continued, in control plots, it was 2.22 and in 50 : 50 ratio plots, it was 4.54. At harvest, the same number of branches was there as we observed at flowering stage in all the treatments with slight differences (Table 3). Earlier reports have shown that PGPR can improve the growth of sweet potato and papaya (Radziah & Zulkifli, 2003 and Shivan & Kumbar, 2019). Wins and Murugan (2010) investigated the influence of a textile mill effluent on the germination and growth of *Vigna mungo* (black gram) seedlings and found that seedling growth was stronger at lower effluent concentrations than in controls, but the growth was slow as waste water concentrations increased. The concentration of nutrients in agro-based industrial effluent can be too high for crops, resulting in over-fertilization, nutrient leaching and yield reduction (Mok *et al.*, 2014). This could explain why the number of shoots in treatment T_5 (RDF + UMFE), in which 100 per cent UMFE was supplied, was lower. Treatment T_6 had a higher number of shoots per plant than T_5, despite supplementing 100 per cent UMFE with beneficial microbes. This could be due to the presence of PGPM, which has the potential to absorb nitrogen and supply ammonical form of nitrogen to the plant. These PGPM are also known to have plant

TABLE 3

Plant height and number of shoots per plant of potato as influenced by different treatments at different growth stages

Treatments	Plant height (cm)			No. of shoots per plant		
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
T1	22.16 ± 1.82 ^{g}	28.07 ± 2.66 ^{e}	33.43 ± 0.87 ^{f}	1.89 ± 0.70 ^{b}	2.22 ± 0.58 ^{b}	2.44 ± 0.12 ^{c}
T2	35.78 ± 1.41 ^{f}	41.82 ± 3.60 ^{d}	49.24 ± 2.16 ^{e}	2.93 ± 0.23 ^{ab}	2.56 ± 0.89 ^{b}	2.55 ± 0.10 ^{c}
T3	39.45 ± 0.81 ^{e}	51.39 ± 2.65 ^{c}	55.63 ± 0.60 ^{c}	3.14 ± 0.57 ^{ab}	3.11 ± 0.38 ^{b}	3.14 ± 0.19 ^{c}
T4	45.67 ± 1.63 ^{c}	50.83 ± 0.59 ^{c}	55.03 ± 0.66 ^{c}	3.11 ± 0.38 ^{ab}	3.89 ± 0.01 ^{ab}	3.92 ± 0.31 ^{b}
T5	43.79 ± 1.31 ^{d}	50.54 ± 1.52 ^{c}	53.52 ± 2.13 ^{d}	3.76 ± 0.21 ^{ab}	3.92 ± 0.23 ^{ab}	3.95 ± 0.12 ^{b}
T6	50.00 ± 0.58 ^{b}	54.50 ± 2.08 ^{b}	58.92 ± 0.66 ^{b}	4.37 ± 0.61 ^{a}	5.11 ± 0.51 ^{a}	5.15 ± 0.21 ^{a}
T7	52.89 ± 1.71 ^{a}	58.89 ± 3.02 ^{a}	64.44 ± 1.18 ^{a}	4.27 ± 0.77 ^{ab}	4.54 ± 0.34 ^{a}	4.78 ± 0.24 ^{a}

T1 - Absolute Control ; T2- RDF (Control) ; T3- RDF + PGPM ; T4- RDF + Jeevamrutha ; T5- RDF + UMFE ; T6- RDF + UMFE + PGPM ; T7- RDF + 50:50 (Water: UMFE) + PGPM; RDF- Recommended Dosage of Fertilizers ; PGPM- Plant Growth Promoting Microbes (N fixer, P solubilizer, *P. fluorescence*) ; UMFE- Untreated Marigold Flower Leachate ; Values in the same column with the same letter are not significantly different (*P = 0.05) ; ± indicates standard deviation value among replicates growth promoting abilities such as phytohormones production, siderophore production and biocontrol activity.

The number of leaves per plant is presented in Table 4. At 30 DAS the number of leaves was significantly higher in treatment 7 *i.e.*, RDF + 50 : 50 + PGPM (19.26) and the lowest was in control plants where only borewell water is given (9.51). At flowering stage the more number of leaves was found in treatment water and UMFE water in the ratio 50 : 50 along with PGPM (109.66) and in the control plots it was 45.31. The higher number of shoots in the respective treatments has resulted in corresponding increase in the number of leaves per plant. The withering impact resulted in a dramatic drop in the quantity of leaves in all treatments during the harvesting stage. Umashankar *et al.* (2019) in a prior experiment employed marigold flower leachate on a field bean crop discovered similar findings. This could be owing to the presence of NH_4^+ and NO_3^-, two ionic forms of nitrogen that help to increase the number of meristematic cells.

At all growth stages, the chlorophyll content in leaves was measured using a SPAD meter in SPAD units and it was discovered that there was a steady decline in chlorophyll content in all the treatments over time (Table 4). Studies conducted by using paper mill effluent for irrigation of wheat crop reported similar results with decrease in chlorophyll content and with increase in effluent concentration (Chaturwedi *et al*., 1995). Residual chemicals in that water, on the other hand, led early stunted growth in plant root systems with limited access to available nutrients. As a result, vegetative development and chlorophyll production was reduced (Li *et al*., 2007).

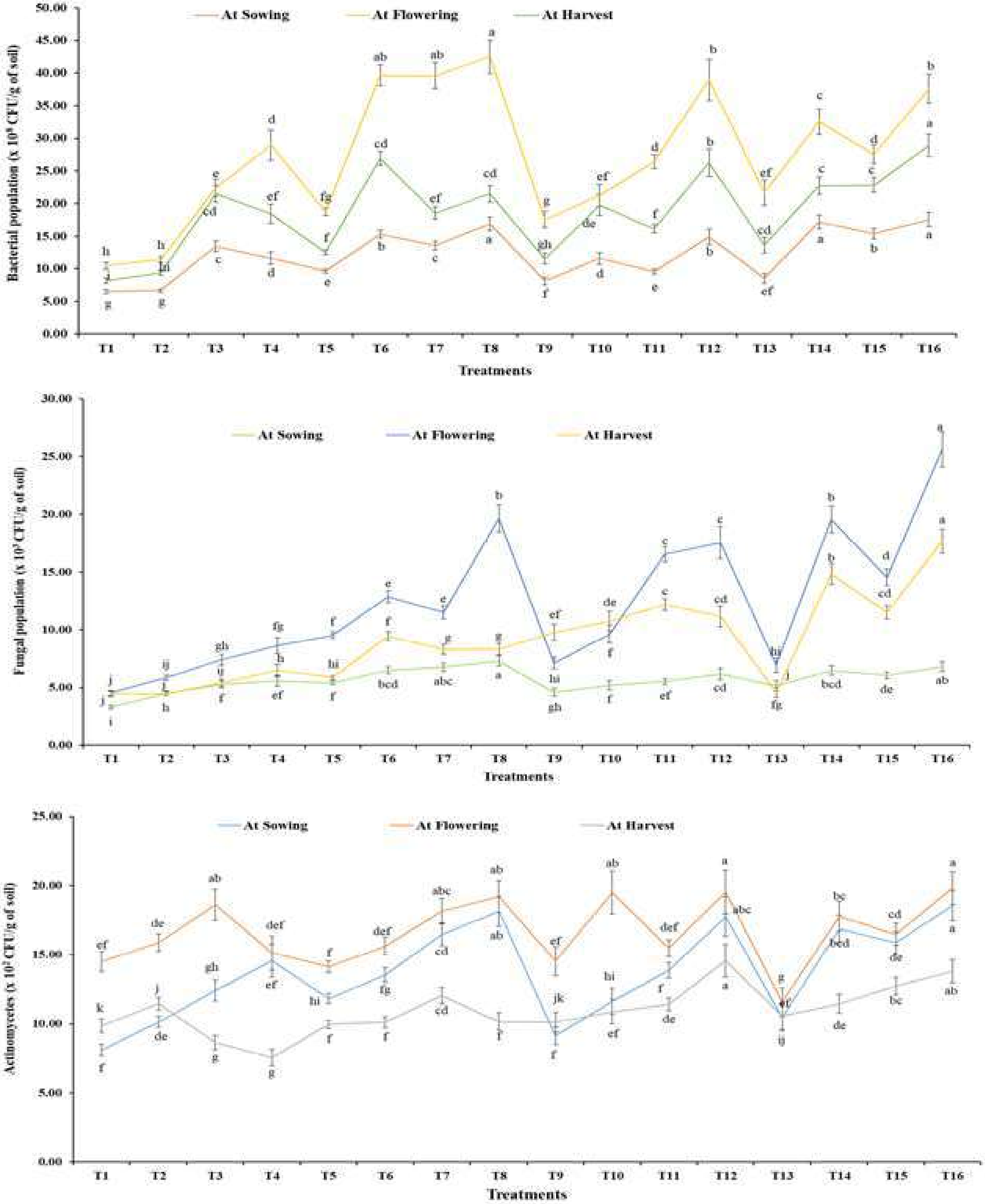
The application of UMFE and PGPM had a significant impact on the microbiological population of the soil. The treatments T_6 (34.86 x 10^8 CFU / gm of soil) and T_7 (35.47 x 10^8 CFU / gm of soil) reported highest bacterial population 30 DAS and consequently, T_7 reported highest population at flowering (42.75 x 10^8 CFU / gm of soil) and at harvest (32.56 x 10^8 CFU / gm of soil). Least bacterial population was noticed in T_1 and T_2 and were on par with each other at all the growth stages. Similar trend was observed in fungal and actinomycetes population with a fungal population of 21.54, 29.58 and 16.78 x 10^3 CFU / gm of soil at 30 DAS, at flowering and at harvest, respectively. Actinomycetes population among all the treatments varied from 5.46 and 26.79 x 10^2 CFU / gm of soil at 30 DAS, 18.92 and 43.75 x 10^2 CFU / gm of soil at the time of flowering, 10.45 and 15.77 x 10^2 CFU / gm of

TABLE 4

Number of leaves per plant and chlorophyll content of potato as influenced by different treatments at different growth stages

Treatments	No. of leaves per plant			SPAD units		
	30 DAS	60 DAS	At Harvest	30 DAS	60 DAS	At Harvest
T1	9.51 ± 0.48 [e]	45.31 ± 2.27 [d]	24.01 ± 1.20 [f]	34.89 ± 2.59 [d]	31.75 ± 3.60 [e]	22.41 ± 1.12 [d]
T2	12.19 ± 0.49 [d]	70.34 ± 2.81 [c]	40.53 ± 1.62 [ef]	39.13 ± 3.54 [d]	35.20 ± 2.47 [e]	21.54 ± 0.86 [d]
T3	14.19 ± 0.85 [cd]	81.53 ± 4.89 [b]	52.53 ± 3.51 [c]	42.56 ± 5.23 [c]	40.16 ± 1.84 [d]	32.12 ± 1.93 [a]
T4	14.81 ± 1.18 [cd]	73.59 ± 5.89 [c]	46.34 ± 3.71 [d]	44.86 ± 4.67 [b]	39.84 ± 1.86 [d]	25.54 ± 2.04 [cd]
T5	14.23 ± 0.43 [cd]	75.81 ± 2.27 [c]	43.69 ± 1.31 [e]	47.93 ± 2.94 [a]	41.55 ± 0.63 [c]	26.42 ± 0.79 [c]
T6	19.23 ± 0.77 [a]	105.06 ± 4.20 [a]	57.37 ± 2.29 [b]	49.24 ± 4.25 [a]	45.04 ± 1.89 [a]	32.42 ± 1.30 [a]
T7	19.26 ± 0.96 [a]	109.66 ± 5.48 [a]	58.88 ± 2.94 [a]	49.70 ± 2.08 [a]	43.74 ± 2.05 [b]	30.21 ± 1.51 [b]

T1- Absolute Control ; T2- RDF (Control) ; T3- RDF + PGPM ; T4- RDF + Jeevamrutha ; T5- RDF + UMFE ; T6- RDF + UMFE + PGPM ; T7- RDF + 50:50 (Water: UMFE) + PGPM; RDF- Recommended Dosage of Fertilizers ; PGPM- Plant Growth Promoting Microbes (N fixer, P solubilizer, *P. fluorescence*) ; UMFE- Untreated Marigold Flower Leachate ; Values in the same column with the same letter are not significantly different (*P = 0.05) ; ± indicates standard deviation value among replicates



*Values with the same letter are not significantly different (*P = 0.05); the standard deviation error bars have been mentioned

Fig. 1 : Soil microbial population of bacterial, fungi and actinomycetes as influenced by different treatments at different growth stages of Potato

soil. There was a gradual decrease in Bacterial, Fungal and Actinomycetes over the crop growth period and at harvest in all treatments (Fig. 1). Supplying plants with nitrogen exceeding their exact requirements with waste water could cause a reduction in economic yield and food nutrient quality (Qadir and Scott *et al*., 2009 and Chen *et al*., 2013) and may affect soil microbial communities (Becerra Castro *et al*., 2015).

The yield parameters of the experiment are presented in Table 5. The number of tubers per plant was significantly higher in the treatment 7 (9.43), where water and UMFE water was applied at the rate of 50:50 along with PGPM and RDF compared to all other treatments. The lower number of tubers per plant was observed in plots treated with RDF along with jeevamrutha (7.50). The tuber yield was significantly higher in treatment 7 in which UMFE + water are applied in 50 : 50 along with PGPM and RDF (19.68 t ha^{-1}) and lower tuber yield was recorded in treatment 1 (5.90 t ha^{-1}). Libutti *et al*. (2018) and Lingaraju, 2019 observed similar findings and they mentioned the reuse of treated agro-industrial waste water for irrigation can be considered as an effective way to cope with agricultural water shortage in the Mediterranean area. In similar fashion soybean yield also increased in the study conducted by Hati *et al*. (2007), the seed yield of soybean in all the distillery effluent treated plots was significantly more than control and was similar to the 100 per cent NPK + FYM treatment. Farhadkhani *et al*. (2018) mentioned that industrial waste water can be a safe, alternative source for leafy and root crops irrigation.

Table 5

Yield parameters of Potato crop as influenced by different treatments

Treatments	Number of tubers per plant	Tuber yield (kg ha^{-1})
T1 (Absolute Control)	7.51 ± 0.38 [b]	5900 ± 295.00 [g]
T2 (RDF (Control))	7.81 ± 0.31 [b]	13160 ± 366.40 [f]
T3 (RDF + PGPM)	7.92 ± 0.48 [b]	15460 ± 867.60 [c]
T4 (RDF + Jeevamrutha)	7.50 ± 0.60 [b]	14680 ± 854.40 [d]
T5 (RDF + UMFE)	6.09 ± 0.18 [c]	14440 ± 403.20 [e]
T6 (RDF + UMFE + PGPM)	8.11 ± 0.32 [b]	17970 ± 718.80 [b]
T7 (RDF + 50: 50 (Water: UMFE)+ PGPM)	9.43 ± 0.47 [a]	19680 ± 984.00 [a]

RDF - Recommended Dosage of Fertilizers ; PGPM - Plant Growth Promoting Microbes (N fixer, P solubilizer, *P. fluorescence*) ; UMFE - Untreated Marigold Flower Leachate ; Values in the same column with the same letter are not significantly different (*P = 0.05); ± indicates standard deviation value among replicates.

Application of untreated marigold flower leachate (UMFE) to the agricultural field, as an amendment, might be a viable option for the safe disposal of this industrial waste with concomitant enhancement in yield and improvement in physical properties of the soil by diluting it by 50 per cent. Several researches have revealed that the irrigation with industrial waste water had no adverse effect on vegetables, ground water or the food chain. Enhancing the leachate with PGPM resulted in better yields as these microbes help in solubilizing the nutrients available in the marigold flower leachate and make it available for plant system.

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