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## OPTIMIZING WASTEWATER REUSE: EFFECTS OF SEWAGE TREATED WATER AND REVERSE OSMOSIS WASTE WATER COMBINATIONS ON GERMINATION AND ITS RELATED ATTRIBUTES IN GREEN GRAM (*Vigna radiata* L.).

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### ABSTRACT

India's increasing population, urbanization and industrialization have intensified pressure on freshwater resources, particularly in agriculture, which consumes the highest proportion of available water. To explore safe alternatives, the present study was evaluated the effects of various combinations of canal water, Sewage Treated Water (STW) and Reverse Osmosis Wastewater (ROWW) on germination and early seedling growth in green gram (*Vigna radiata* L.). A laboratory experiment was conducted at the Vanavarayar Institute of Agriculture, Pollachi, Tamil Nadu, using the between-paper (BP) method. Eight irrigation treatments were tested including individual and combined sources Viz., 100% Canal Water (T1), 100% STW (T2), 100% ROWW (T3), 75% Canal Water + 25% STW (T4), 50% Canal Water + 50% STW (T5), 25% Canal Water + 75% STW (T6), 50% Canal Water + 50% ROWW (T7), and 50% STW + 50% ROWW (T8). Results revealed that T4 (75% CW + 25% STW) recorded the highest germination percentage (95%), root length (11.0 cm), shoot length (17.0 cm), vigour index I (2660), and vigour index II (13.82), followed by T1 (100% CW). Similar trend was observed in germination dynamics. In contrast, T3 (100% ROWW) consistently showed the lowest performance across germination and its related attributes, indicating that undiluted RO reject water is unsuitable for seed germination and early growth stages. The findings demonstrate that partial substitution of canal water with treated wastewater can enhance seedling performance, thereby supporting sustainable water management strategies in agriculture.

**Keywords:** Canal water, Sewage treated water, RO waste water, seed germination, Vigour index

### Introduction

India, with its rapidly growing population, faces significant challenges in balancing natural resource use and sustainability. While Population growth in India is growing by geometric rate, food production advances more slowly in arithmetic progression, leading to increasing gap between food demand and supply. At the same time Rapid industrialization and urban expansion mounting pressure on fresh water resources, especially in water scarce regions. The scarcity of freshwater is a critical constraint for sustainable agriculture development, especially in a country where agriculture remains a primary livelihood and accounts for the highest share of

water consumption (Wang et al., 2017). Currently, India generates approximately 72,368 million litres per day (MLD) and has the capacity to treat about 37% of it, and only 28% is actually treated (CPCB, 2024). Tamil Nadu is one of the most water stressed states, generates 3,938 MLD wastewater with just 17% being treated and reused. Notably, Chennai alone contributes to nearly 58 per cent of the total reuse. These figures reflect a significant untapped potential for safe wastewater recycling, particularly in the agricultural sector.

Groundwater, which fulfills 80 to 90 percent of drinking water and irrigation needs in India, is under severe stress due to overexploitation and contamination. Declining

water tables, coupled with pollution from industrial and domestic effluents, threatens the agricultural productivity as well as public health. As conventional water sources become unreliable especially in arid and semi-arid regions, farmers rely on alternative sources for irrigation such as treated wastewater, industrial effluents, canal water, and even Reverse osmosis (RO) reject water (Qadir *et al.*, 2007).

The reuse of treated wastewater offers multiple benefits for plants and crops (Angelakis *et al.*, 1999). It not only conserves freshwater but also recycles essential plant nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and trace elements, which can enhance soil fertility and crop yield (Pathak *et al.*, 1999; Lubello *et al.*, 2004; Nath *et al.*, 2009). Similarly, RO reject water—typically discarded during household or industrial water purification—contains salts and minerals at levels that may still be suitable for irrigation when diluted or blended appropriately. For every litre of RO-purified water, about three litres of wastewater are produced, representing a substantial volume of potentially reusable water. With the increasing stress on limited water resources these sources help mitigate water scarcity, but they often contain elevated levels of heavy metals and salts, which could impair soil health and pose food safety risks (Singh *et al.*, 2009).

Limited studies were existed on the combined effects of multiple irrigation water sources (e.g., sewage treated wastewater and RO waste water with Canal water) on germination and seedling Vigor we need to assessing the effects of different irrigation water combinations on germination and seedling vigor which is essential for promoting sustainable reuse practices. Green gram (*Vigna radiata*), a widely cultivated fast growing legume in tropical and subtropical regions, is not only valued for its nutritional content and soil-enriching properties but also serves as an effective test crop for environmental monitoring. Its short life cycle, sensitivity to soil and water changes make it suitable for studies.

Based on above dynamics, the present investigation was carried out at Vanavarayar Institute of Agriculture, Pollachi, Tamil Nadu, to optimize the effect of varying proportions and combinations of groundwater, sewage water and RO reject water on germination and early growth of green gram.

### Materials and Methods

A laboratory experiment was carried out at Agronomy lab of Vanavarayar Institute of Agriculture, Manakkadavu, Pollachi, to assess the effects of different irrigation water combinations on seed germination and

its related attributes in green gram (*Vigna radiata* L.).

### Collection of Water samples

For germination studies, three water samples were collected from three distinct sources in our campus. Canal Water (CW) was collected from a nearby field irrigation canal with continuous flow. Sewage Treated Water (STW) was collected from the outlet of the campus sewage treatment plant. Reverse Osmosis Wastewater (ROWW) was obtained from the reject outlet of an RO unit in our campus after flushing for 1–2 minutes. These samples represent varied water qualities to assess their suitability for seed germination.

### Methods of preparation of irrigation water samples

Water samples used for irrigation treatments were prepared by collecting Canal water, sewage treated water and RO wastewater were collected in clean, sterilized plastic containers. For combination treatments, the collected water samples were measured accurately by using calibrated measuring jars and mixed in the required ratios (e.g., 50:50, 75:25) just before application to ensure uniformity. All water samples were stored in shaded conditions and used immediately for germination tests to preserve the physicochemical properties.

### Setup for the investigation of the experiment

The Germination test was performed by using between-paper (BP) techniques prescribed by ISTA, 2015. Germination was counted daily and total seedling length was recorded at the end of the study. The data recorded on the daily count of Germinants were used to calculate various germination indices as given below.

### Heavy metal Analysis

Heavy metal analysis of different water sources was analyzed by atomic absorption Spectrometer.

### Measurement and Analysis of data

The treated seeds were evaluated for the following seed and seed quality characters. Number of germinant was counted daily from the onset of germination up to 7 days thereafter. From the daily count of germinant the following parameters were computed. Germination percentage, shoot length, root length and Vigour index I {Germination % X total seedling length (cm)} and Vigour index II {Germination percent X total dry weight of seedling} Abdul-Baki and Anderson, 1973.

The other germination parameters were 1) Germination Energy (GE), 2) Germination Value (GV), 3) Emergence Energy Value (EEV) and 4) Germination Relative Index (GRI)

**Table 1:** Heavy metal Analysis of 100 % concentration of RO waste water, Canal Water and Sewage treated Water.

| Metals   | RO water | Canal water | STW   |
|----------|----------|-------------|-------|
| Chromium | BDL      | BDL         | BDL   |
| Lead     | BDL      | BDL         | BDL   |
| Nickel   | 0.0007   | BDL         | BDL   |
| Cadmium  | BDL      | BDL         | 0.001 |

**Germination energy (GE) (Maguire, 1962)**

Germination energy (GE) was calculated after Maguire (1962) by the formula

$$GE = \frac{X_1}{X_2} + \frac{X_2 - X_1}{Y_2} + \dots + \frac{X_n - X_{n-1}}{Y_n}$$

Where,

X<sub>n</sub> is the number of germinants on the n<sup>th</sup> counting date,

Y<sub>n</sub> the number of days from sowing to the n<sup>th</sup> count.

**Germination value (GV)**

Germination value as defined by Czabator (1962) is the integral of final mean daily germination percentage (MDG) and peak value (PV). Final MDG MDG is mean daily germination calculated as the percentage of full-seed germination at the end of the test divided by the number of days to the end of the test. PV is peak value, or the maximum quotient derived from all of the cumulative full-seed germination percentages on any day divided by the number of days to reach this percentages.

**Emergence Energy Value (EEV)**

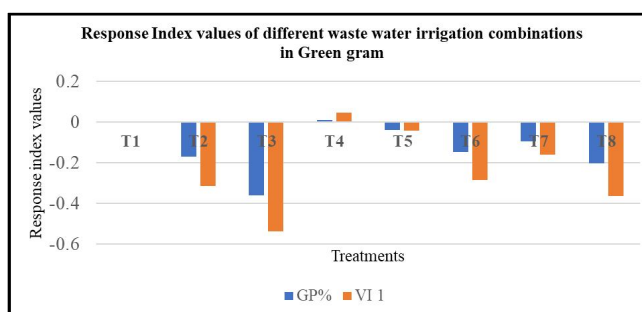
Emergence Energy Value (EEV) is the highest value obtained when the germination percentage on a day is divided by the number of days since test begun, when that germination percentage was reached Bahuguna *et al.* (1987).

**Germination Relative Index**

Germination Relative Index (GRI) was computed after Sreevatsava and Sareen (1972) by the formula,

**Table 2:** Effect of Various combination of Irrigations on Germination percentage, root length, shoot length and Vigour index in green gram.

| Treatments              | G.P(%) | Root length (cm) | Shoot length (cm) | VI(I)  | VI(II) |
|-------------------------|--------|------------------|-------------------|--------|--------|
| T1 - 100 % CW           | 94     | 10.5             | 16.5              | 2538.0 | 13.45  |
| T2 - 100% STW           | 78     | 7.8              | 14.5              | 1739.4 | 6.16   |
| T3 - 100% ROWW          | 60     | 5.6              | 14.0              | 1176.0 | 4.20   |
| T4 - 75% CW + 25% STW   | 95     | 11.0             | 17.0              | 2660.0 | 13.82  |
| T5 - 50% CW + 50% STW   | 90     | 10.5             | 16.5              | 2430.0 | 9.72   |
| T6 - 25% CW + 75% STW   | 80     | 8.2              | 14.5              | 1816.0 | 8.32   |
| T7 - 50% ROWW + 50% CW  | 85     | 9.0              | 16.0              | 2125.0 | 10.45  |
| T8 - 50% ROWW + 50% STW | 75     | 7.5              | 14.0              | 1612.5 | 8.18   |
| S.Ed value              | -      | 0.36             | 0.59              | 76.75  | 0.35   |
| C.D Value (0.05)        | -      | 0.77             | 1.25              | 162.70 | 0.75   |

**Fig. 1:** Response Index values of different waste water irrigation combinations in green gram.

$$[\Sigma X_n (h - n)]$$

Where,

X<sub>n</sub> is the number of germinants at n<sup>th</sup> count;

h, the total number of counts

n, the count number.

**Response Index (RI)**

The magnitude of inhibition versus stimulation in bioassay was compared through response index (RI) (Richardson & Williamson, 1988) determined as follows:

if T > C, RI = 1 - (C/T)

if T = C, then RI = 0

if T < C, then RI = (T/C) - 1

Where,

T is the treatment mean and C is the control mean. A negative RI reflects the proportional disparity in output (germination, radicle length or plumule growth) of test crop in the treatment relative to output in the control.

**Statistical analysis**

All the data on observations were recorded and subjected to ANOVA as suggested by (Gomez and Gomez, 1984) for completely Randomized Block Design. The significant critical differences were worked out at Five percent probability level. The non-significant treatment differences were denoted by NS.

**Table 3:** Response Index values for various irrigation combinations on green gram.

| Treatments              | G.P(%) | Shoot length (cm) | Root length (cm) | VI(I)  | VI(II) |
|-------------------------|--------|-------------------|------------------|--------|--------|
| T1 - 100 % CW           | +0.00  | +0.00             | +0.00            | +0.00  | +0.00  |
| T2 - 100% STW           | -0.17  | -0.121            | -0.257           | -0.314 | -0.554 |
| T3 - 100% ROWW          | -0.36  | -0.151            | -0.466           | -0.536 | -0.695 |
| T4 - 75% CW + 25% STW   | +0.01  | +0.02             | +0.045           | +0.045 | -0.07  |
| T5 -50% CW +50% STW     | -0.04  | +0.00             | +0.0             | -0.042 | -0.296 |
| T6 - 25% CW + 75% STW   | -0.15  | -0.121            | -0.219           | -0.284 | -0.393 |
| T7 - 50% ROWW + 50% CW  | -0.09  | -0.03             | -0.142           | -0.162 | -0.243 |
| T8 - 50% ROWW + 50% STW | -0.20  | -0.151            | -0.285           | -0.364 | -0.408 |

## Results and Discussion

### Effect of different irrigation combinations on germination and its related parameters

The present study explored the effect of different combinations of wastewater irrigation treatments on germination and early seedling growth parameters in green gram (*Vigna radiata*). The results are presented in (Table 2 & 3 and Fig. 1.). Significant variations were observed among the treatments and showed mixed values of response index in terms of germination percentage, root and shoot lengths, and seedling vigour indices (Vigour Index I and II).

Among different irrigation treatments, T<sub>4</sub> treated with 75% Canal Water + 25% Sewage-Treated Water recorded the highest germination percentage (95%), root length (11.0 cm), shoot length (17.0 cm), Vigour index I (2660) and Vigour index II (13.82) and it was statistically on par with T<sub>1</sub> when treated with 100% Canal Water showed the values of (94 % germination, 10.5 cm root length, 16.5 cm shoot length and Vigour index I and II of 2538 and 13.45 respectively. The observed increase in seedling performance at lower concentrations of sewage-treated water may be attributed to the presence of essential nutrients that promote early seedling development. This finding aligns with Divya *et al.*, (2015) reported low sewage effluent concentrations (10–30/ %), enhanced the germination percentage, root and shoot lengths and seedling vigour index in green gram (*Vigna radiata*), red gram (*Cajanus cajan*), and paddy (*Oryza sativa*).

Conversely, T<sub>3</sub> (100% RO waste water) exhibited the lowest performance, with 60% germination, root length (5.6 cm), Shoot length (14.0 cm), and lowest vigour index I (1176) and II (4.20). These results clearly indicate the potential inhibitory effects of RO reject water on early seedling development when applied without dilution. These findings were similar with Sandamali *et al.*, (2019) reported Germination percentage and seedling growth decreased with the increasing concentrations of RO reject water. They noted that combination of 40% of RO

rejected water with 60% pure water showed better performance in *Amaranthus* and maize. While green gram and cabbage responded well at lower concentrations 20% of RO rejected wastewater and 80% pure water. The negative impact at higher concentrations is likely due to elevated salt content and other contaminants in RO reject water and which may lead to osmotic stress and toxicity during early seedling growth stages.

### Effect of different irrigation combinations on germination energy, germination value, germination relative index and emergence energy value

The present study explored the effects of irrigation treatments on germination dynamics in green gram, focusing on Germination Energy (GE), Germination Relative Index (GRI), Germination Value (GV), and Emergence Value (EV). These parameters are critical indicators of seed vigor and early seedling establishment. As per ISTA (2006), Germination energy reflects the percentage of fast germinating seeds and also determines the viability and vigour. Similarly, Germination value is an index of combining speed and completeness of seed germination Czabator, 1962 and is related to survival of seedlings Djavanshir and Pourbeik, 1976. while Emergence Value provides a composite measure of the potential for uniform and early seedling emergence.

The results presented in (Table 4) revealed marked differences among the treatments. The highest values

**Table 4:** Effect of various combination of Irrigations on germination related attributes in green gram.

| Treatments              | (GE)  | (GRI) | (GV)    | (EEV)       |
|-------------------------|-------|-------|---------|-------------|
| T1 - 100 % CW           | 22.16 | 136   | 1181.71 | 22          |
| T2 - 100% STW           | 15.79 | 99    | 668.57  | 15          |
| T3 - 100% ROWW          | 15.51 | 99    | 514.28  | 15          |
| T4 - 75% CW + 25% STW   | 20.86 | 123   | 1131.42 | <b>19</b>   |
| T5 -50% CW +50% STW     | 17.83 | 112   | 925.71  | 18          |
| T6 - 25% CW + 75% STW   | 14.32 | 89    | 594.28  | 13          |
| T7 - 50% ROWW + 50% CW  | 16.50 | 101   | 922.85  | 19          |
| T8 - 50% ROWW + 50% STW | 19.58 | 118   | 814.28  | <b>19</b>   |
| <b>S.Ed value</b>       | 0.66  | 6.84  | 30.94   | <b>0.65</b> |
| <b>C.D Value (0.05)</b> | 1.40  | 14.51 | 65.60   | 1.38        |

across all germination dynamics were recorded in T1 (100% Canal Water), with Germination Energy of 22.16, Germination Relative Index of 136, Germination Value of 1181.71 and Emergence Energy Value of 22, indicating that superior quality of canal water favors for optimal germination and early seedling emergence and it serves as bench mark for comparing the other irrigation treatments. Treatment T4 (75% Canal Water + 25% Sewage-Treated Water), was statistically on par with T1 which also showed relatively high values (GE: 20.86, GRI: 123, GV: 1131.42, EEV: 19), suggesting that a small proportion of treated wastewater can be integrated without significantly compromising seed performance when canal water is scarce. These findings are aligned with Jadaun *et al.*, (2020), who found that low concentrations of treated wastewater (20–40%) could enhance seed germination and early growth in crops like green gram and cabbage.

T8 (50% ROWW + 50% STW) also exhibited relatively high GE (19.58), GRI (123) and a moderate GV (814.28). This implies that while initial germination was rapid, subsequent seedling development might have been hindered by the combined influence of RO waste water and Sewage treated wastewater.

On the other hand, the poorest germination responses were recorded in T6 (25% CW + 75% STW) showed reduced values (GE: 14.32, GV: 594.28, EV: 13) and T3 (100% RO Wastewater), with the lowest GV (514.28), GE (15.51), and EV (15), highlighting the higher concentration of waste water may inhibit seed vigor and uniform emergence. This might be due to presence of some inhibitory substances, dissolved salts and other chemical constituents hinder the enzymatic activities essential for early seed germination and seedling development. These findings were similar with Salian *et al.*, (2018) who observed highest concentration of RO reject water significantly reduced germination percentage and related attributes in crops such as chickpea, Maize and pigeon pea.

## Conclusion

From the investigation of different waste water irrigations two conclusions can be drawn. Treatments with higher proportions of canal water, particularly 100% canal water (T1) and 75% canal water + 25% sewage-treated water (T4), consistently supported superior germination parameters, including germination energy, value, and seedling vigor. Increased concentrations of RO reject water or untreated/poorly treated wastewater resulted in reduced seedling performance.

These findings indicate that partial substitution of canal

water with treated wastewater (up to 25%) can be a viable and sustainable irrigation strategy without compromising seedling quality. However, the direct use of undiluted wastewater should be avoided, particularly during the critical stages of germination and early seedling development.

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