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EFFECTS OF ANTIBIOTIC STRESS ON GERMINATION OF RADISH (*RAPHANUS SATIVUS* L.) AND TURNIP (*BRASSICA RAPA SUBSP.* RAPA) CROPS

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Abstract

Antibiotics are beneficial in combating disease-causing agents. However, their excessive usage has led to environmental contamination through wastewater, manure, and effluents from livestock facilities, classifying them as emerging contaminants. To assess the phytotoxic effects of antibiotic stress, erythrocin and doxycycline (0, 5, 10, and 15 mM) were applied to radish (*Raphanus sativus* L.) and turnip (*Brassica rapa subsp.* rapa.) in a petri plate germination experiment, conducted at the Physiology Laboratory, Department of Botany, The Women University, Multan. Both crops exhibited distinct germination responses under antibiotic stress. Turnip (var. PT) showed complete germination inhibition (0%) at 15 mM erythrocin, whereas radish (var. R Manu) retained 77% germination at the same concentration. Antibiotic stress significantly reduced radicle length more than germination rates. Erythrocin caused a 17% reduction in radish (var. R 40), 3% in radish (var. R Manu), and 92% in turnip (var. TG), while doxycycline led to reductions of 50% in (var. R 40, 92% in (var.TG), and 77% in (var. PT). Erythrocin demonstrated greater phytotoxicity than doxycycline, highlighting species- and concentration-dependent effects.

Key words: Doxycycline, Erythrocin, Germination index, Radish, Turnip

INTRODUCTION

Antibiotics are critical for maintaining both human and animal health, but their overuse over the past two decades has resulted in widespread environmental contamination of soil and water systems (Grenni *et al.*, 2018). Approximately 30–90% of administered antibiotics are excreted unmetabolized, entering agroecosystems via manure, wastewater irrigation, or biosolids (Zhou *et al.*, 2022), These contaminants pose risks to plant physiology, soil microbiota, and human health through bioaccumulation in crops (Rocha *et al.*, 2021a; Tadić *et al.*, 2021b)

Root and tuber crops, such as radish and turnip, are nutrient-rich staples with high global demand. However, their broad-leaf morphology and tuberous roots may enhance antibiotic uptake (Lal *et al.*, 2021). Studies report variable antibiotic absorption across species: lettuce and carrot accumulate amoxicillin (27.1 ng/g) and tetracycline (20.2 ng/g) in edible parts (Christou *et al.*, 2019), while pea roots exhibit tetracycline-induced oxidative stress (Margas *et al.*, 2019). Notably, germination and early seedling growth are sensitive bioindicators of antibiotic toxicity (Pan and Chu, 2016).

Hillis *et al.* (2011) researched on the effects of 10 antibiotics at concentrations ranging from 1 to 10,000 g/L on seed germination and root elongation in carrot (*Daucus carota*), alfalfa (*Medicago sativa*), lettuce (*Lactuca sativa),* and demonstrating that there were no toxic effects on germination even at the highest concentrations. Similarly, Eluk *et al.* (2016) explained difference in the germination pattern of different vegetables varieties with different antibiotic stress at varying concentration such as enrofloxacin (1 mg/L), penicillin (0.004 mg/L), and oxytetracycline (10 mg/L) on soybean, enrofloxacin (0.1 mg /L on sunflower, and effect of penicillin (4 mg/L) and oxytetracycline (100 mg/L) on corn. However, there have been the situations of quite high contamination reported in the literature, such as pig slurry with up to 235 mg/L fresh weight of sulfadiazine and 66 mg/L of tetracycline, which could directly harm plant germination and root development (Timmerer *et al.*, 2020). As soil is the medium for plants and continuous addition of antibiotics in soil can affect the germination of seeds. These two vegetables can be used in a variety of ways, including boiled, raw, fermented, baked, and cooked form, they were chosen for the direct interaction of antibiotics (Lal *et al.*, 2021). Tadić *et al.* (2021a) and Christou *et al.* (2019) suggested that, how much pollution is absorbed by plants depends on the type of vegetable and species.

Earlier studies found minimal germination effects at high antibiotic concentrations (Hillis *et al*., 2011), recent evidence suggests species-specific sensitivity. For instance, Timmerer *et al*. (2020) observed complete germination inhibition in *Sinapis alba* at 235 mg/L sulfadiazine, emphasizing the need for crop-specific assessments. This study evaluates the effects of erythrocin and doxycycline on radish and turnip germination, addressing gaps in understanding antibiotic impacts on tuberous vegetables.

METHODOLOGY

Two winter crops radish and turnip were selected to evaluate the impact of antibiotic stress on seed germination. Seeds of two varieties of radish (var. R40, and var. R Manu) and turnip var. TG (Turnip Golden) and var. PT were obtained from Nuclear Institute of Agricultural and Biology, Faisalabad.

Experimental Design: A randomized block design with three replicates was employed. Seeds of both radish (*Raphanus sativus*) varieties (var. R40, Manu) and turnip (*Brassica rapa*) varieties (var. TG, PT) were treated with erythrocin (250 mg/L) and doxycycline (100 mg/L) at 0, 5, 10, and 15 mM concentrations respectively

Solution Preparation: Antibiotic solutions were prepared using molecular weights (MW) to calculate molarity. For example, 1 mM erythrocin (MW 733.9 g/mol) required 0.733 g/L. Solutions were diluted to 250 mL to minimize waste.

Petri plates of 9*9 cm were used and base was covered by the two layers of Whatman filter paper. Ten seeds of each variety of both crops were placed on wet filter paper (wetting done with10 mL of each respective solution). In accordance with the ASTM (American Society for Testing and Materials) standard germination methodology. The Petri plates were placed in the dark with their lids to promote dormancy. Petri plates were kept at ~~a~~ temperature of 27°C in total darkness. To keep and maintain moisture in petri plates, slight sprinkling was done on daily basis. To record the germination data, germinated seed with radical reaches up to 1.0 mm considered as germinated seeds. Germination data recorded for 7 days. With the help of a ruler measured radical length of germinated seeds in each petri plates. After the 7th day the germination index (GI) was calculated by using radical length.

Germination index

The seven-day germination data was used to compute GI. Calculate relative seeds germination (RSG), relative roots elongation

(RRE), and then GI by using the formula below (Tam and Tiquia, 1994).

$$RSG = \frac{\text{no.of seeds germinated in extract}}{\text{no.of seeds germinated in control}} \text{ x } 100$$

$$RRE = \frac{\text{mean root elongation in extract}}{\text{mean root elongation in control}} \text{ x } 100$$

$$GI = \frac{\textbf{\% seed germination x \% root elongation}}{\textbf{100\%}}$$

Statistical analysis

The data was statistically analyzed using COSTAT and MS Excel. Applied three way completely randomized ANOVA by using COSTAT. The least significant difference (LSD) was determined at 5% level of significance.

RESULTS

Present results indicated that the first day of germination showed a maximum decrease at 5 mM (50%) in turnip *TG*, radish *R Manu* (67%) treated with erythrocin and in radish *R40* (80%), and radish *var. R Manu* (11%) treated with doxycycline. While radish *var. R40* showed nearly equal rate of germination at different concentration. Turnip *PT* with erythromycin showed germination only at 5 mM while no germination was observed in case of turnip *PT* treated with doxycycline. However, radish *var. R Manu* (22%) and turnip *TG* (100%) treated with doxycycline showed increased in germination at 10 mM and Results from ANOVA suggested significant ($p \leq 0.001$**)** relation between crops while non-significant (p = ns**)** relation was observed between varieties, treatment, and interactions between crops x varieties, crops x treatment, varieties x treatment, and crops x varieties x treatment.

The second day germination showed maximum decreased at 15 mM in turnip TG (88%) and radish Manu (17%), while no germination had occurred in turnip PT treated with erythrocin but radish R40 (19%) showed maximum decreased at 10 mM However, radish 40 (56%), turnip TG (87%) and turnip PT (76%) showed maximum decreased at 15 mM with doxycycline. While radish Manu showed maximum increase (7%) at 15 mM however radish 40 (25%), and turnip golden (33%) at 5 mM Results from analysis of variance showed significant ($p \leq 0.001$**)** relation between crops and treatment while interaction between crops x treatment, and crops x varieties showed significant relation at level of ($p \leq 0.05$**).** In 3rd to 5th days of germination except turnip PT with erythrocin and radish Manu with doxycycline all varieties showed maximum decrease at 15 mM in case of turnip PT with erythrocin germination was totally inhibited at 15 mM while nearly equal germination pattern was observed at 5 mM and 10 mM. However, germination was increased in radish 40 (18%) and turnip golden (13%) at 5 mM while radish Manu at 10 mM (14%) and 15 mM (7%). Result showed significant ($p \leq 0.001$**)** relation between crops, treatment and interaction between them. Same sequence of germination was observed from 6th and 7th days of germination with significant ($p \leq 0.001$**)** relation between crops, treatment, interaction between crops x treatment, and crops x varieties ($p \leq 0.05$**)**.

Root length, relative root elongation, and germination index indicated maximum decreased at 15 mM However, root length and related parameters nearly equal to zero in turnip golden at 15 mM while completely inhibited at 15 mM (erythrocin) in turnip PT.

Relative seed germination showed a reduction at 15 mM in all varieties except radish Manu with doxycycline. Radish Manu showed an increase in seeds germination however turnip 40 at 5 mM This indicated that varieties (Radish Manu and turnip 40) treated with doxycycline showed tolerance in germination of seeds also doxycycline was less effective than erythrocin.

Statistical Significance: ANOVA revealed significant differences between crops ($p \leq 0.001$) and treatments ($p \leq 0.001$). Interactions (crop × treatment) were significant for radicle length ($p \leq 0.05$).

DISCUSSION

The germination process can be speed up or slowed down by antibiotics, despite the fact that this has no impact on ultimate germination percentages (Marcelo Pedrosa Gomes *et al.*, 2019). According to Minden *et al.* (2017) antibiotics can delay germination. Germination rates are typically severely impacted by concentrations that are excessive or by reduced biomass, which could have a detrimental impact on yield.

Germination Sensitivity: Turnip (PT) exhibited greater sensitivity to erythromycin than radish, aligning with species-specific trends reported by Christou *et al*. (2019). The complete inhibition of PT at 15 mM contrasts with Hillis *et al.* (2011), who found no germination effects at 10,000 µg/L antibiotics in lettuce, suggesting tuberous crops may face higher risks.

Radicle Inhibition: The pronounced reduction in radicle length (Figure 2A) supports Pan and Chu (2016), who identified root elongation as a more sensitive endpoint than germination. This aligns with the skotomorphogenesis stage, where rapid radicle growth increases antibiotic susceptibility (Luo *et al.*, 2020a; Luo *et al.*, 2020b)

Erythromycin's greater phytotoxicity than doxycycline may relate to its molecular structure, which impedes mitochondrial protein synthesis in plants (Krupka *et al.*, 2022). Conversely, doxycycline's moderate effects mirror its weaker binding to plant ribosomes (Rocha *et al*., 2021). Timmerer *et al*. (2020) reported 100% germination inhibition in *Sinapis alba* at 235 mg/L sulfadiazine, consistent with *PT*'s response to erythrocin. Pan and Chu (2016) observed 20–50% root inhibition in lettuce at 1 mg/L antibiotics, contrasting with our >70% reductions, likely due to higher concentrations (15 mM ≈ 11,000 mg/L erythromycin).

Doxycycline, however, reduced root length while increasing germination in radish 40 and radish *Manu* at concentrations of 15 mM and 5 mM, respectively. Although antibiotics had no effect on germination rates, they discovered that increasing antibiotic concentrations decreased root elongation. Present study results revealed that there is a substantial relationship between different varieties of radish and turnip treated with different concentrations of antibiotics, contrary to suggestion that there is no relationship between the various varieties of crops. The findings confirmed that seed germination is substantially less responsive to antibiotics than radicle elongation (Luo *et al.*, 2019). Cotyledon and axis are parts of the embryo, whereas hypocotyl and radical are parts of the axis. The stage of plant skotomorphogenesis when the radical is the quickly growing organ and antibiotic-sensitive occurs when seeds germinate (Luo *et al.*, 2019). According to Rocha *et al.* (2021b) the response of plants to antibiotic combinations can vary depending on the chemical properties of the antibiotics in addition to being concentration dependent.

CONCLUSIONS

Erythrocin imposes greater phytotoxicity on germination and radicle growth than doxycycline, with turnip *(*PT*)* being highly sensitive. Turnip (PT) showed complete inhibition (0%) at 15 mM, while radish (R Manu) maintained 77% germination with Erythrocin. While with Doxycycline, germination decreased by 50% in *R40* and 77% in turnip PT at 15 mM. Erythrocin reduced radicle length by 88% in turnip TG and 76% in turnip PT at 15 mM Doxycycline caused milder inhibition (56% in R40, 33% in turnip TG).

DECLARATION

It is declared that the paper entitled "Effects of Antibiotic Stress on Germination of Radish (*Raphanus sativus* L.) and Turnip (*Brassica rapa* ***subsp.*** rapa) Crops" to be submitted to Journal Plantarum for the publication. This paper has not been submitted to any other Journal for the award of publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest in this publication**.**

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AUTHOR'S CONTRIBUTION

Hussan Bano Conceived the idea and design the experiment. Neelam Shahzadi performed the experiment. Akasha and Maimoona analyze the data and help to draft the first draft of MS. Hussan Bano revised the MS.

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Table 1: Mean squares from ANOVA for the data of seed germination of radish and turnip grown under antibiotic stress for seven days.

Source	df	1st day	2nd day	3rd day	4th day	5th day
Crops	1	58.594***	416.667***	301.042***	276.760***	240.667***
Varieties	1	0.510ns	0.042ns	3.375ns	3.010ns	2.042ns
Treatments	3	1.510ns	37.236***	57.167***	57.066***	73.903***
Crops x Varieties	1	1.760ns	20.167*	8.167ns	8.760ns	13.5ns
Crops x Treatment	3	1.566ns	12.972*	26.708***	28.094***	36.528***
Varieties x Treatment	3	0.705ns	1.903ns	1.375ns	0.955ns	0.292ns
Crops x Varieties x Treatment	3	0.455ns	0.583ns	2.222ns	2.594ns	1.417ns
Error	80	0.939ns	4.487	3.879	3.848	3.955
Source	**df**	**6th day**	**7th day**	**df**	**RL**	
Crops	1	204.167***	228.167***	1	80.797***	
Varieties	1	0.375ns	0.167ns	1	0.034ns	
Treatments	3	81.028***	79.708***	3	109.632***	
Crops x Varieties	1	22.042*	15.042*	1	0.042ns	
Crops x Treatment	3	39.917***	43.111***	3	16.351***	
Varieties x Treatment	3	0.181ns	0.556ns	3	0.021ns	
Crops x Varieties x Treatment	3	1.125ns	0.708ns	3	0.021ns	
Error	80	3.8	3.504	79	0.495	
Total	95			94		

ns = non-significant; *, * significant at 0.05 and 0.001 probability levels, respectively**

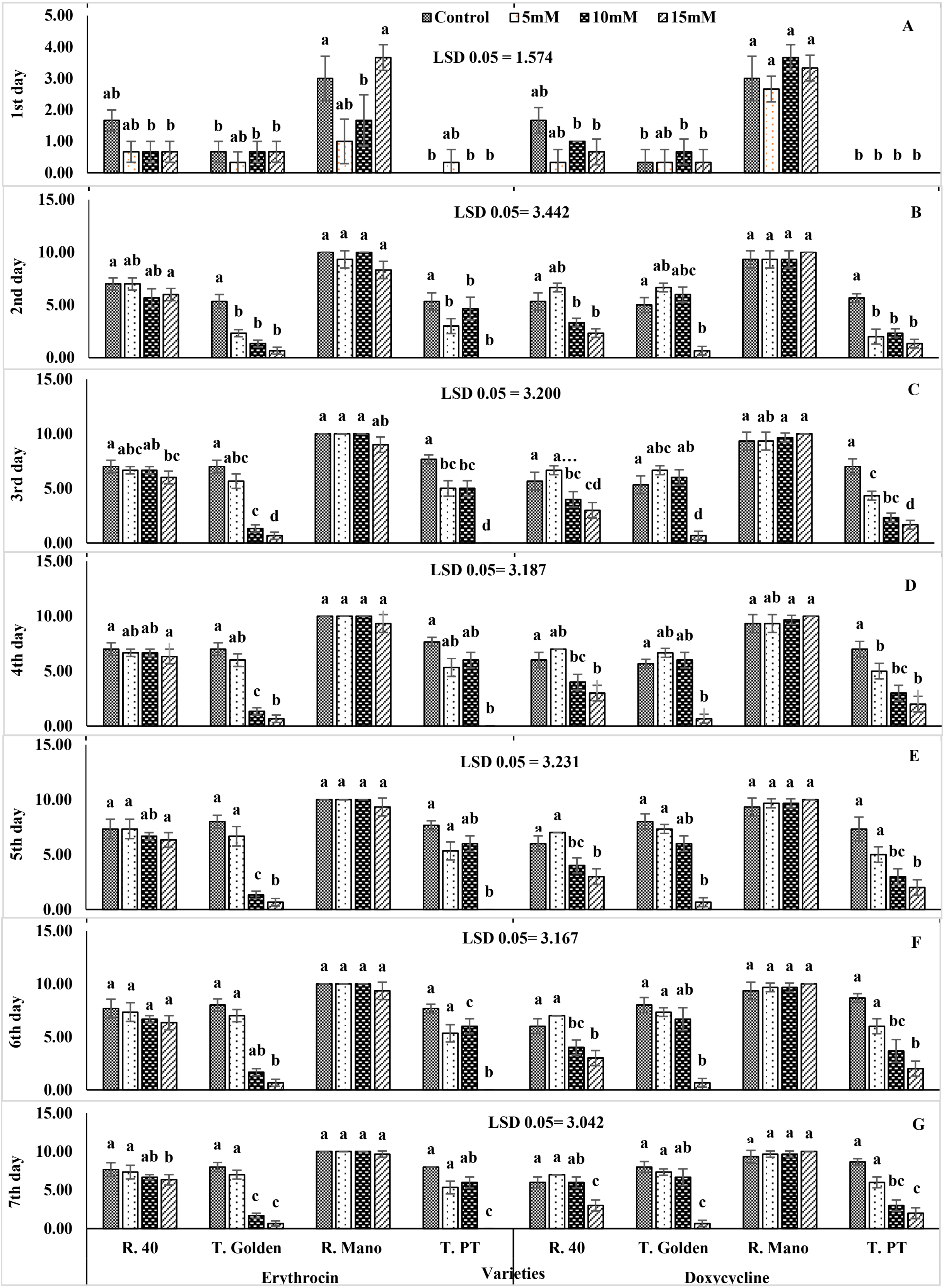

Figure 1. Germination data of two crops radish (var. R 40 and var. R Manu) and turnip (var. T Golden and var. PT) under two antibiotic (Erythrocin and Doxycycline) stress (mean ± S.E, n =3, small letters (a-d) indicating least significant difference between mean value of treatment).

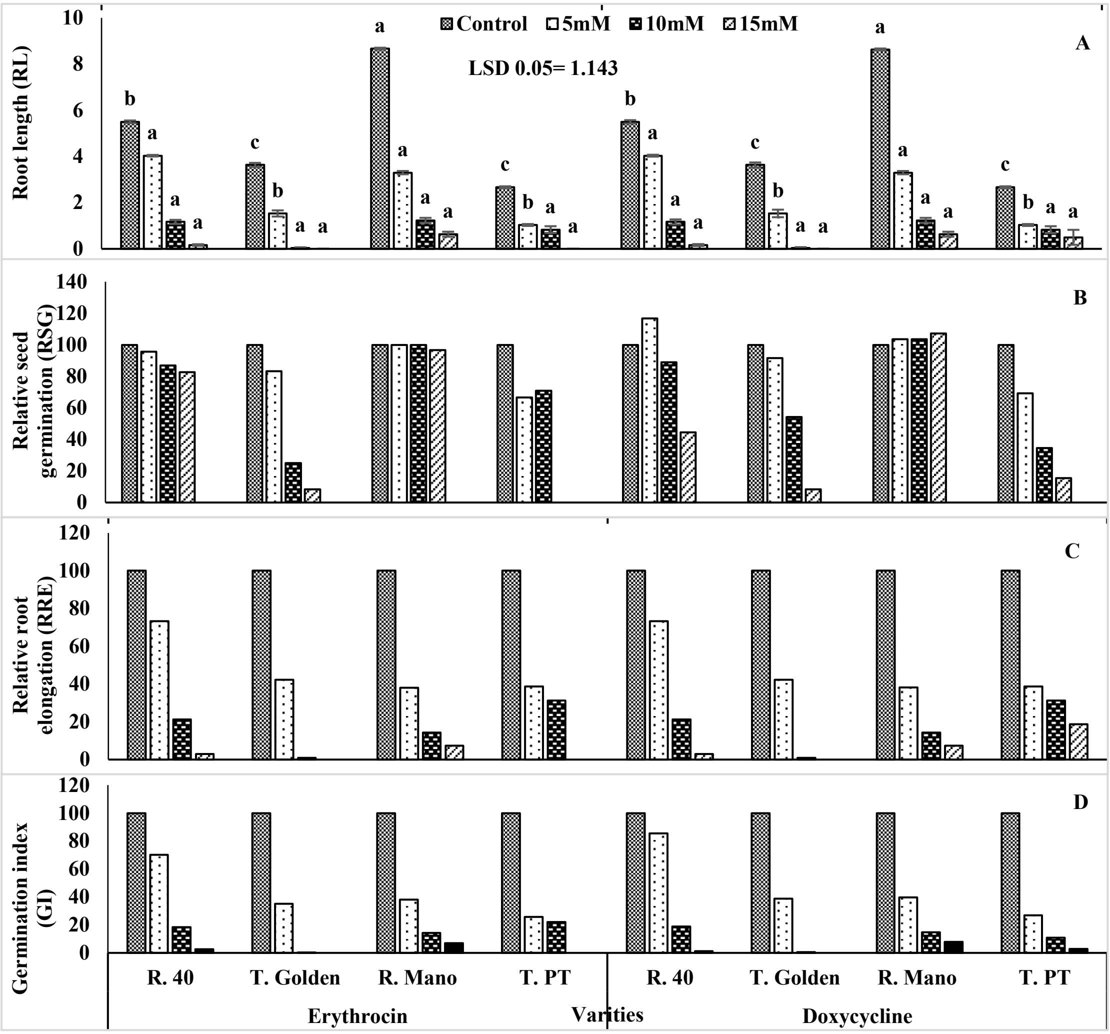

Figure 2. Root length (A) Relative seed germination (B) Relative root elongation (C) Germination index (D) of two crops radish (var. R 40 and var. R Manu) and turnip (var. T Golden and var. PT) under two antibiotic (Erythrocin and Doxycycline) stress (mean ± S.E, n =3, small letters (a-c) indicating least significant difference between mean value of treatment).

Figure 3. Seed germination experiment of radish, and turnip under antibiotic stress in petri plates