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Assessing the nature of transmission, key soil enzyme activities and population dynamics of *Ralstonia solanacearum* (Smith), in different ginger-based intercropping approaches

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Abstract

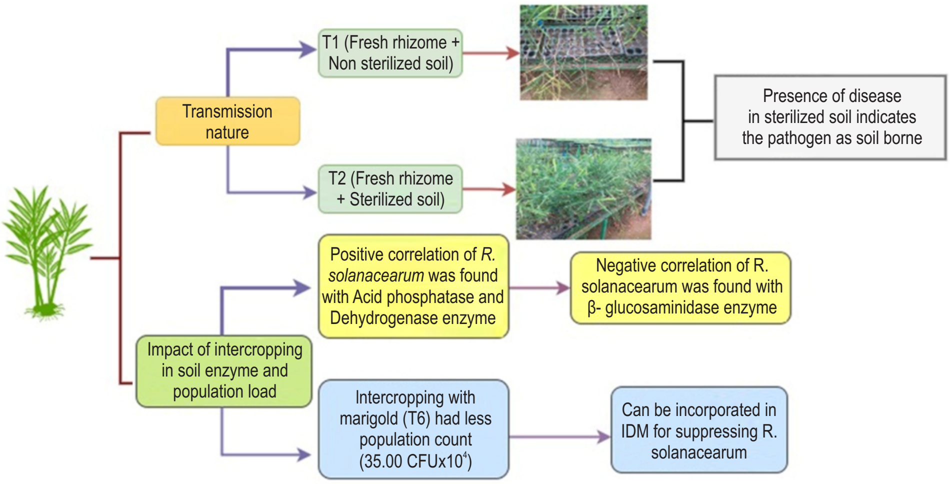
Aim: To check the seed borne nature and impact of different ginger-based intercropping system on key soil enzymes and population dynamics of *Ralstonia solanacearum* (Smith) under organic management practices.

Methodology: The study aimed to determine the transmission nature of *R. solanacearum* taking two treatments (T_1: Fresh rhizome +non-sterilized soil and T_2: Fresh rhizome + sterilized soil) with seven replications using healthy single ginger bud and the effect of six different ginger based intercropping system on key soil enzyme and population dynamics of *R. solanacearum.*

Results: The results obtained indicated the presence of wilted and rotted plants in sterilized soil (T_2) suggesting the involvement of infected rhizomes in disease spread. Furthermore, a significant positive correlation was found between the population density of *R. solanacearum* and both Acid phosphatase and Dehydrogenase enzymes. In contrast, a notable negative correlation was observed between the population density of *R. solanacearum* and β-glucosidase enzymes. Also, intercropping with marigold gave good results in reducing the population density as compared to other treatments under study.

Interpretation: The findings suggest *R. solanacearum* as a rhizome borne pathogen beside soil born and intercropping with marigold can be effective in suppressing the population load, thereby reducing the disease intensity.

Key words: Intercropping, Population dynamics, Phytopathogen, *Ralstonia solanacearum*, Rhizome, Soil enzymes



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Introduction

Intercropping, a sustainable agricultural practice, involves cultivating two or more crops simultaneously on the same piece of land. In recent years, the exploration of intercropping techniques has gained significant attention in enhancing crop productivity and managing plant diseases. Among the various crops, ginger stands out as a valuable commodity due to its medicinal properties and culinary significance. However, the prevalence of *Ralstonia solanacearum*, (Yabuuchi *et al.,* 1995), a devastating soil-borne pathogen causing bacterial wilt or green disease in ginger plants, poses a considerable threat to its cultivationin Kerala and the North-Eastern region of the country, especially in Sikkim and West Bengal (Sarma and Anandaraj, 2000). The organic practice of growing ginger in this region mostly in hills, have become one of the important challenges for managing the pathogen (Yonzone *et al.,* 2023). Due to devastating lethal nature (Yabuuchi *et al.,* 1995), broad host range (Denny and Hayward, 2000) and worldwide distribution (Elphintone, 2005) *R. solanacearum* is also considered as the second most damaging phytopathogen in the world (Liu *et al.,* 2009; Mansfield *et al.,* 2012).

Soil enzymes play a crucial role in the intricate matrix of soil health, intimately linked to physical properties, organic matter content and microbial activity. Their response often mirror changes in the soil environment, makes them dynamic indicators of soil health and capable of providing early warnings of shifts (Dick *et al.,* 1996). This sensitivity underscores the global emphasis on optimizing soil nutrient utilization and minimizing reliance on inorganic fertilizers by fostering more efficient cropping systems (Zhang and Shen, 1999). The concept of intercropping encompasses not only the intricate interactions among roots, soil and individual plants, but also encompasses the synergistic interplay among the plant community, soil, microorganisms and their environment (Zhang *et al.,* 2002). This agricultural practice has gained significant recognition for its adept utilization of soil nutrients, land resources, water, and solar radiation, surpassing the efficiency of monocropping systems (Rowe *et al.,* 2005; Dhima *et al.,* 2007; Walker and Ogindo, 2003; Xu *et al.,* 2008; Awal *et al.,* 2006; Rodrigo *et al.,* 2001).

Intercropping exerts profound influence on the mobilization of major nutrients such as nitrogen, phosphorus, potassium, thereby enhancing their acquisition and facilitating improved enzymatic activity within the rhizosphere (Wasaki *et al.,* 2003; Khan *et al.,* 2014). The assessment of transmission dynamics, soil enzyme activities, and population dynamics of *R. solanacearum* in different ginger-based intercropping approaches has emerged as a critical area of research. The success of reducing the bacterial population load and suppressing the wilt disease has been reported beneficial in cabbage-based crop rotation (Yonzone *et al.,* 2022). Furthermore, the major impact of cabbage biofumigation has been reported not only on different soil enzyme at active tillering stage, but also on reducing the population load of the pathogen.In this context, understandingof how various intercropping methods affect the key soil enzymes and population dynamics of the pathogen is essential for developing sustainable disease management strategies and improving ginger yield. Therefore, the present investigation was conducted with the aim to delve into the intricate relationship between *R. solanacearum*, soil enzymes and ginger-based intercropping systems.

Materials and Methods

The research was carried out at the Regional Research Station (Hill Zone) of Uttar Banga Krishi Viswavidyalaya, Kalimpong, West Bengal, India, for two consecutive seasons (2021 and 2022). The experiments were carried out in two parts, first to determine the transmission nature of *R. solanacearum* and second to assess the effect of different intercropping system on major soil enzymes and population dynamics of *R. solanacearum.*

Determination of transmission nature of *R. solanacearum:* The rhizome of the ginger variety *Gorubathaney* which is commonly grown in the hill regions of West Bengal was used as the test material. The ginger rhizome was planted using single bud technique (1-2cm size) in two (02) treatments; T_1 (Fresh rhizome + Non-sterilized soil) and T_2 (Fresh rhizome + Sterilized soil), with seven (07) replications. A total of 50 plants were planted in each pot tray for the trial. The soil was sterilized in autoclave at 121 °C for 20 min (Berns *et al.,* 2008). Percent disease incidence was scored every fifteen days, specifically 115 days after sowing, (Horita and Tsuchiya (2001).

Assessment of different intercropping system on major soil enzymes and population dynamics of *R. solanacearum:* To prepare the experimental plot, the land was ploughed twice using an indigenous plough and subsequently leveled manually. Soil incorporation involved the addition of FYM @ 20 t ha^{-1} during the final land preparation. The cultivation process was done organically as per recommended practices without using any synthetic fertilizers in a Random Block Design (Yadav *et al.,* 2017). The experiment consisted of four replications, with each plot measuring 3 m × 1 m. The ginger variety, Gorubathaney, was used with six treatment combinations (T_1- Ginger + Soyabean; T_2- Ginger + Cow Pea; T_3- Ginger + Okra; T_4- Ginger + Marigold; T_5- Ginger + Maize; T_6- Ginger Sole Crop) involving crops commonly grown in the hill zone during different seasons. The enumeration of the *R. solanacearum* population was conducted at active tillering stage following the protocol of Kelman (1954) in specific media (Triphenyl Tetrazolium Chloride) at 30±2ºC. Colonies were counted using a plate counter and multiplied by appropriate dilution factor to determine the number of colonies formed in the original sample.

Estimation of Acid phosphatase: The acid phosphatase activity was assessed according to the standard procedure given by Samuel *et al.* (2010). Acetate buffer (pH 5.0) was added to the reaction mixture, which comprised 2.5 g of soil, 2 ml of toluene (used as an antiseptic), 10 ml of buffer solution and 10 ml of 0.5% substrate solution. A control was maintained with the reaction mixture excluding either soil or substrate solution. Subsequently, the reaction mixture was incubated at 37°C for 2 hr. The phenol content released from the substrate due to phosphatase activity was read at 614 nm on a spectrophotometer, employing a colorimetric reaction to differentiate between phenol and 2,6-dibromoquinone-4-chloroimide. Phosphatase activity was quantified and expressed as mg phenol g^{-1} soil 2 hr^{-1}.

Estimation of Dehydrogenase activity: Dehydrogenase activity was determined following the standard protocol described by Tabatabai (1994). One milliliter of 3% aqueous 2,3,5-triphenyltetrazolium chloride solution was added to soil, followed by the addition of deionized water to each sample. The samples were incubated at 37°C for 24 hr. After incubation, each sample was washed and filtered by adding methanol in 10 ml aliquots until the reddish color disappeared. The filtrate was later diluted to 100 ml with methanol, and the color intensity was read at 485 nm wavelength.

Estimation of β-glucosaminidase: The standard method was employed to determine the β-glucosaminidase activity given by Parham and Deng (2000). In this method, 0.1 M acetate buffer (pH 5.5) and 10 mM ρ-nitrophenyl-N-acetyl-β-D-glucosaminide (ρNNAG) solution were added to soil samples. Following this, the mixture was incubated at 37°C for 1 hr. The reaction was stopped by adding of 0.5 M calcium chloride and 0.1 M tris (hydroxymethyl) amino methane (THAM) buffer (pH 12). The resulting solution was filtered, and the color intensity was read at 420 nm wavelength.

Statistical analyses: For the transmission study, two treatments with seven replications each were used in the pot experiment. The field experiment (intercropping study) followed a randomized block design (RBD) with four replications for each treatment. Percentage data, such as disease incidence, were arcsine-transformed prior to analysis to stabilize variances. Statistical analysis was conducted using ANOVA to evaluate the significance of treatment effects and interactions between treatments. Mean comparisons were performed using the least significant difference (LSD) test at a 5% significance level. Statistical analysis was carried out using SPSS (version 25), following the methods described by Gomez and Gomez (1984). Additionally, the correlation coefficients between soil enzyme activity and the population density of *Ralstonia solanacearum,* as influenced by the ginger-based crop intercropping system, were analyzed using SPSS (version 25).

Results and Discussion

The perusal of data from Table 1 vividly demonstrate notable differences in the impact of the treatment on bacterial wilt and rhizome rot in ginger plants. The incidence of wilting and rotting in T_1 treatment was recorded 96.57% and 90.00%, while in T_2, it was 74.86% wilting and 12.00% rotting (Fig. 1). These results signify a considerable level of significance for both treatments, with t-values of 0.002 ($P < 0.05$) for wilting and 0.000 ($P < 0.01$) for rotting percentage. The observation of wilted and rotted plants in sterilized soil suggests the involvement of infected rhizomes in pathogenicity, thereby contributing to the incidence of bacterial wilt disease. The seed-borne nature of *R. solanacearum* in ginger rhizomes was first demonstrated by Thammakijjawat *et al.* (2006) through real-time PCR, which detected the pathogen in asymptomatic rhizomes. This finding underscores the role of latent infections in pathogen dissemination. Similarly, Kifelew *et al.* (2015) confirmed the pathogen's ability to colonize various hosts as a seed-borne agent, highlighting its epidemiological importance. Behera *et al.* (2020) elaborated on its role in ginger bacterial wilt, demonstrating how infected planting material significantly contributes to disease outbreaks. Additionally, Kumar and Sarma (2004) characterized *R. solanacearum* from ginger and provided insights into its pathogenicity and persistence in plant tissues. These studies collectively emphasize the pathogen's dual nature—both seed-borne and soil-borne, and the critical need for stringent seed health management to mitigate disease incidence.

The impact of various ginger-based intercropping methods with commonly grown crops in the hill zone on significant soil enzymes, namely Acid phosphatase, Dehydrogenase, and β-glucosidase, alongside the population density of the bacterial wilt-causing pathogen *R. solanacearum,* was documented at active tillering stage of the crop growth and is presented in Table 2. The findings reveal substantial variations in different enzymes influenced by distinct ginger-based intercropping systems. The maximum Acid phosphatase activity 464.00μg pNP g^{-1} hr^{-1} (464.00) was observed in T_6 treatment, while the minimum activity

Table 1: *In-vitro* evaluation of ginger rhizome through single bud technique in disseminating the bacterial wilt causing pathogen *R. solanacearum*

Treatments	Wilting	Rotting	Ooze test
T_1 (Fresh rhizome + Non sterilized soil)	96.57 (77.19)	90.00 (12.00)	+
T_2 (Fresh rhizome + Sterilized soil)	74.86 (60.19)	12.00 (17.06)	+
Mean		60.19	17.63
SE(m)±		2.25	2.04
T value		0.002*	0.000**

Figure in the parenthesis are arc sign transformed values; *=significant at 5% level and **= significant at 1% level; += Positive Ooze test

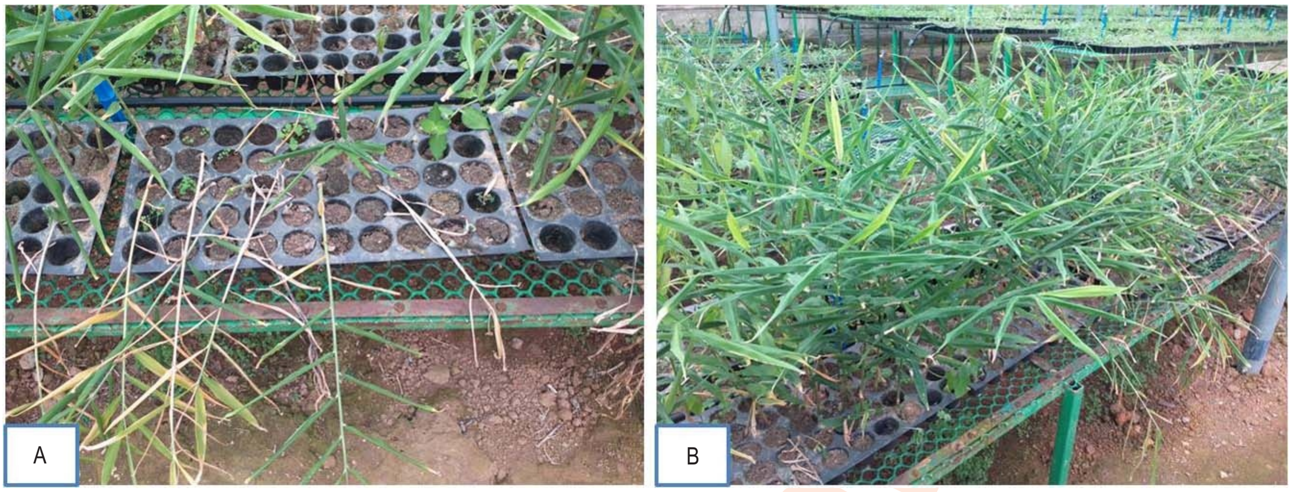

Fig. 1: Symptoms produced in both the treatments (A). T_1: Fresh rhizome + Non-sterilized soil and (B). T_2 : Fresh rhizome + Sterilized soil).

Table 2: Effect of different intercropping system on major soil enzymes and population dynamics of *R. solanacearum*

Treatments	**Acid phosphatase (μg pNP g^{-1} hr^{-1})**	**Dehydrogenase (μg TPF g^{-1} hr^{-1})**	**β- glucosidase (μg pNP g^{-1} hr^{-1})**	**Population at Active tillering stage (CFU x10^4)**
T_1: Ginger+soyabean	457.00	2.30	36.24	46.75
T_2: Ginger+Cowpea	449.00	3.05	34.82	59.00
T_3: Ginger+Okra	432.00	3.11	35.96	66.25
T_4: Ginger+marigold	427.75	2.28	34.96	35.00
T_5: Ginger+Maize	417.25	2.65	37.34	44.25
T_6: Ginger+sole	464.00	1.52	29.46	84.00
C.D (%)	14.45	0.19	1.93	12.06
SE(m) ±	4.75	0.06	0.64	3.97

417.00 µg pNP g^{-1} hr^{-1} was noted in T_5 treatment. The maximum dehydrogenase activity 3.11 µg TPF g^{-1} hr^{-1} was observed in T_3 treatment, closely, followed by T_2 treatment (3.05 µg TPF g^{-1} hr^{-1}). Additionally, the highest β-glucosidase activity 37.34 µg pNP g^{-1} hr^{-1} was recorded in T_5 treatment whereas the lowest activity 29.46 µg pNP g^{-1} hr^{-1} was found in T_6 treatment. Furthermore, the bacterial population (CFU X$10^{\wedge 4}$) during the active tillering stage exhibited the highest count in T_6 treatment (84.00 CFU X$10^{\wedge 4}$), followed by T_3 treatment (66.25 CFU X$10^{\wedge 4}$). Conversely, the lowest population during this stage was observed in T_4 treatment (35.00).

Soil enzymatic activities are crucial for determining the soil microbial activity and fertility. The enzyme phosphatase plays a crucial role in converting organic phosphorus into inorganic phosphate, making it directly accessible to plants and soil organisms. Under phosphorus-stressed conditions in organically grown intercrops, increased secretion of acid phosphatase from plant roots can enhance phosphate solubilization and remobilization. This heightened secretion of acid phosphatase correlates positively with the *R. solanacearum* population in ginger-based intercropping systems. Mono-cropping however has been shown to negatively impact plant growth and increase the prevalence of various soil-borne diseases (Hiddink *et al.,* 2009). Additionally, intercropping has been found to influence the microbial biomass, activity, abundance, composition and structure of antagonistic microorganisms (Zhang *et al.,* 2018; Li *et al.,* 2016a; Li and Wu 2018). The T_2 and T_3 treatments, which combined ginger with cowpea and okra, showed balanced enzymatic activities and reduced pathogen populations, highlighting the benefits of legume intercropping in enriching soil nitrogen and microbial diversity (Li *et al.,* 2016). Additionally, diverse crop combinations create a complex root architecture that fosters beneficial microbial consortia, reducing pathogen establishment (Wu *et al.,* 2018; Tian *et al.,* 2019).

The significant effect of ginger rotation with cabbage and marigold in minimizing the disease incidence and increasing the yield has also been reported by Yonzone *et al.,* (2021); Li *et al.,* (2020). These results align with the findings that diverse cropping systems improve soil health and disrupt pathogen life cycles by altering the microbial community structure (Yang *et al.,* 2020).

Table 3: Correlation coefficient between soil enzymes and population density of *R. solanacearum* as influenced by ginger based crop intercropping system

	Acid phosphatase (µg pNP g^{-1} hr^{-1})	**Dehydrogenase (µg TPF g^{-1} hr^{-1}**	**β- glucosidase (µg pNP g^{-1} hr^{-1})**	**Population at Active tillering stage (CFU x10^4)**
Acid phosphatase	1.00			
Dehydrogenase	0.266	1.00		
β- glucosidase	-0.528**	-0.337	1.00	
Population at Active tillering stage	0.571**	0.786**	-0.553**	1.00

**. Correlation is significant at the 0.01 level (2-tailed) ; *. Correlation is significant at the 0.05 level (2-tailed)

Ginger rotation with marigold, in particular, may leverage the biofumigation effect of marigold roots, which release thiophenes known to suppress soil-borne pathogens, including *R. solanacearum* (Bais *et al.,* 2006).

The analysis of the correlation coefficients between the population density of *R. solanacearum* and various soil enzymes including Acid phosphatase, Dehydrogenase and β-glucosidase was done and is presented in the Table 3. The findings revealed a noteworthy positive correlation between the population density of *R. solanacearum* and both Acid phosphatase (0.571) and Dehydrogenase enzymes (0.786). Conversely, a significant negative correlation was observed between the population density of *R. solanacearum* and β-glucosidase enzymes (-0.553). The dehydrogenase enzyme serves as a vital indicator of biological activity in the soil, its increased presence in intercropping system with ginger may possibly be due to the presence of organic matter, and the application of farmyard manure which also showed a positive correlation with the *R. solanacearum* population. The positive correlation of dehydrogenase enzyme with soil activity has also been reported by Chu *et al.* (2007). Moisture during the trial period could also influence increased dehydrogenase enzyme levels, as its presence depends on soil air-water conditions. Glucosidase enzymes, prevalent in soil, play a pivotal role in catalyzing the breakdown of various compounds in plant debris.

Similar to dehydrogenase enzymes, β-glucosidase enzymes are positively correlated with soil organic matter content. However, in this study, a negative correlation was observed between β-glucosidase and *R. solanacearum* population, which might be due to increase in the antagonistic microbial biomass favored by higher soil organic carbon content and elevated β-glucosidase activities (Garcia *et al.,* 2000; Ekenler *et al.,* 2000). The management of soil-borne pathogens due to diversity and richness of bacterial community mostly influenced by marigold intercropping system has been documented by Li *et al.* (2020). They further reported the maximum presence of bacterial genus, *Lysobacter* in intercropping as compared to mono-cropping system which may have influenced the *R. solanaceaous* population. The antimicrobial effect of *Lysobacter* in managing various soil-borne diseases, such as Phytophthora blight of pepper, sugar beet and damping-off of cucumber due to the secretion of various antibiotics such as reduction of zoospore motility or mycelial growth by antibiotic compounds, or to the capacity to inhibit *Pythium* by competition, has been reported by Folman *et al.* (2003) and Islam *et al.* (2005).

Furthermore, the ability of β-glucosidase activity to influence microbial interactions has been explored by Trasar-Cepeda *et al.* (2008), who suggested that this enzyme contributes to soil suppression of pathogens by enhancing microbial nutrient cycling. Similarly, the positive effects of dehydrogenase activity in promoting disease resistance through enhanced microbial functional diversity were observed by Lee *et al.* (2022), emphasizing its potential role in sustainable disease management practices. These findings underscore the importance of intercropping systems, especially those involving marigold, as a sustainable approach to suppress *R. solanacearum* and promote a balanced microbial ecosystem.

Overall, the results align with the broader literature, including studies by Yonzone *et al.* (2021, 2023), which highlight the significant impact of intercropping on suppressing *R. solanacearum* populations and improving soil enzyme activity. The negative correlation between *R. solanacearum* population and dehydrogenase and β-glucosidase activities in this study further validates these findings. Future research should focus on identifying specific microbial species promoted by intercropping systems to develop targeted biocontrol strategies for managing bacterial wilt.

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