Evaluation of Biocontrol-BasedFormulationsAgainst Late Leaf Spot and Rust in Groundnut

ABSTRACT

This study investigated the efficacy of various biocontrol-basedformulationsagainst late leaf spot (LLS)(*Phaeoisariopsispersonata*) and rust (*Puccinia arachidis*) in groundnut. Pot culture and field experiments were conducted during the *Kharif* season (June-September) of 2022. Disease incidence of both late leaf spot and rust was significantly lower in groundnut plants treated with a combination of biocontrol-basedformulationscompared to control. The most effective eco-friendly treatment included a combination of *Trichoderma asperellum*, *Pseudomonasfluorescens*, fortified lignite fly ash and Annamalai mixture. This combination significantly reduced lesion frequency and Percent Disease Index (PDI)for both diseases at 50, 70 and 90 DAS, compared to other treatments. The combination of biocontrol-based formulationsalso significantly increased the pod yield. Furthermore, the same treatment increased the induction of defense enzymes peroxidase (2.22fold), polyphenol oxidase (1.28 fold) and phenylalanine ammonia-lyase (6.25 fold). While Carbendazimeffectively reduced disease incidence, it was surpassed by the biocontrol-based modulein terms of both disease control and yield.These findings suggest that biocontrol-basedformulationscan be a sustainable approach for managing LLS and rust diseases in groundnut cultivation.

Keywords: Leaf spot, rust**,** defense enzyme, biocontrol-based module, groundnut

1. INTRODUCTION

Groundnut (*Arachishypogaea* L.), is often hailed as the "king of oilseeds." Globally, Indiahas the largest cultivation area,ranking second in global production. In India, groundnut production in the *kharif* season is 68.57 lakh tonnes with an average productivity of 1,562 kg/ha (IOPEPC, 2023). Groundnutfosters soil health through symbiotic nitrogen fixation with Rhizobia bacteria, promoting sustainable agricultural practices.Groundnut cultivation faces a formidable challenge from fungal diseases like late leaf spot (LLS), rust,Alternaria blight, stem rot, dry root rot and collar rot [1,2].Among these, LLSdisease caused by *Phaeoisariopsis personata* and rust disease caused by *Puccinia arachidis* pose the most significant threats. These widespread fungal pathogens inflict severe damage on groundnut crops.In most cases, these two pathogens occur together and hamper groundnut production[3]. In India, these diseases have been documented to cause yield losses exceeding 70%, significantly impacting groungnutproductivity[4]. Leaf rust alone could cause yield reduction reaching up to 65%, especially in areas with high rainfall [5]. The detrimental effects extend beyond yield reduction, as the quality of groundnut seeds is also compromised by these fungal infections.

In groundnut cultivation, conventional disease management practices for controlling LLS and rust often rely on chemical fungicides. Their indiscriminate use has led toconcerns about environmental and human health risks[1].Environmental contamination, disruption of ecological balance due to harm to beneficial soil microbes, and potential health risks to humans and non-target organisms are some of the concerning consequences associated with chemical fungicide application[6,7].The emergence of eco-friendly alternatives presents a promising solution for sustainable groundnut production[8]. Antagonistic microorganisms offer a particularly effective approach for managing these fungal diseases. These beneficial microbes can act against pathogens through multiple mechanisms, including competition for resources, production of antibiosis compounds, predation and parasitism [9]. Additionally, some antagonists have the potential to induce systemic resistance within the plant itself[10].

These mechanisms offer a multifaceted approach to disease control, potentially mitigating the need for harmful chemical fungicides.

This work explores use of antagonist microorganisms, as a sustainable and effective disease management strategy againstthe detrimental fungal pathogens, *P. personata* and *P. arachidis* in groundnut.

2. MATERIALS AND METHOD

2. 1 Survey of late leaf spot and rust disease incidence in groundnut

Field surveys were employed to assess the prevalence and severity of groundnut LLS and rust diseases in Cuddalore district. Villages with a history of groundnut cultivation were targeted to capture representative data. Ten locations were chosen for fixed-plot surveys. Within each plot, the number of groundnut plants exhibiting symptoms of LLSand rust disease were recorded alongside the total number of plants observed.

2.2Inoculum culture of *P. personata* and *P. arachidis*

Naturally infected leaves with LLS and rust disease symptoms were collected from fields within the Cuddalore district. These diseased leaves served as the inoculum source for *P. personata* and *P. arachidis*. The inoculum was maintained through pot culture containing groundnut plants.

2.3Assessment of disease severity

Groundnut seeds (cv. VRI 2) were grown in pots (20 cm dia, 5 plants/pot) for 30 days within a polyhouse. The potting medium consisted of a 2:1:1 ratio of field soil, farmyard manure (FYM) and sand. The plants were then inoculated with a spore suspensionmixture of *P. personata* and *P. arachidis*. The spore suspension was prepared by following the protocol of Subrahmanyam *et al.*(11).To achieve optimal infection conditions, an alternating wet and dry period was implemented following inoculation. The inoculated plants were maintained at 24 ± 2°C. Each treatment group within the glasshouse experiments comprised 15 plants with three replicates. Disease severity of both *P. personata* and *P. arachidis* were assessed using disease incidence and lesion frequency following the methods established by Subrahmanyam *et al*. (11).

Quantification of lesion incidence in response to various treatments for combined LLS and rust disease [3].

Lesion frequency (LF) = Number of lesion or pustules/cm^2leaf area

Disease scoring was done with modified 9-point (1-9) scale [11].

$$\text{Percent diseases index (PDI)\%} = \frac{\text{Sum of individual rating}}{\text{Total no. of leaves observed}} \times \frac{100}{\text{Maximum grade}}$$

2. 4 Preparation of biocontrol based formulations.

Aliquots of bovine urine, bovine dung, ovine dung, poultry litter, and neem cake were obtained at a conc. of 100% (w/w) and homogenously mixed in a 1:1:1:1:1 ratio to prepare Annamalai mixture. The resulting organic amendment was filtered and subsequently diluted to 20%, 40%, 60%, and 80% (v/v) using distilled water at the time of application[12].

The antogonists,*T.asperellum* and *P. fluorescens*, were cultivated in Potato Dextrose broth and King's B brothrespectively. Class F lignite fly ash (LFA), obtained from Neyveli Lignite Corporation, Neyveli, India, was used as the carrier material. One kilogram of LFA was mixed thoroughly with 10 grams of carboxymethyl cellulose (CMC). The LFA-CMC mixture was then autoclaved for two consecutive days to ensure proper sterilization.To 1000 g of sterilized LFA-CMC carrier,400 ml of bacterial suspension, containing approximately 8×10^9 CFU/mL, were separately added. 20 ml of molasses was then incorporated into the mixture, followed by thorough mixing. The fortified LFA was shade-dried for 2 h under sterile conditions to remove excess moisture. The final product, designated as fortified lignite

fly ash, was packaged in polyethylene bags and stored at 28°C ± 2°C. To monitor the viability, the population of antagonistic strains within the fortified LFA was periodically evaluated using the serial dilution technique[13].

2. 5Evaluation ofantogonists based formulations against LLS and rustunder pot culture condition

The experiment included 10 treatments with three replications, conducted fromJune to September 2022 at the Department of Plant Pathology, Annamalai University. Previously isolated and available biocontrol agents (BCAs), specifically *T.asperellum*(PP348038) and *P. fluorescens*(PP348041) were evaluated for their ability to suppress the targeted pathogens. Fifteen kilograms of sterilized topsoil from a groundnut field were filled in 45 x 30 cm earthen pots. Groundnut seeds of a susceptible cultivar (VRI-2) were surface sterilized using 3% NaOCl solution for 30 sec, followed by two washes with sterile water. Five seeds were sown per pot. Biocontrol-based components including these BCAs, fortified lignite fly ash (FL-ash) and Annamalai mixture were tested against LLS and rust. The treatments include talc formulation of *T.asperellum* and *P. fluorescens* (2×10^8 CFU g^{-1}) as seed treatments (10 g/kg) and soil drench after sowing, FL-ash as seed treatment (10 g/kg) and soil drench (40 kg/ha equivalent), Annamalai mixture as a seed treatment (10 ml/kg) and foliar spray (20 L/ha equivalent). Spore suspensions of *P. personata* and *P. arachidis* (10^8 spores/ml) were sprayed on plants in the evening at two-time points with a four-day interval, starting 30 days after sowing (DAS). Agar plate technique was employed to confirm the absence of seed-borne pathogens before sowing. Disease incidence and severity were likely assessed throughout the experiment.

2. 6Field evaluation of Biocontrol based strategy against *P. personata* and *P. arachidis*

A field experiment was carried outin an endemic field from June to September, 2022in Sivapuri village, Cuddalore, Tamil Nadu. Groundnut cv. VRI-2 was sown in 33 x 13 feet plots. The biocontrol-based components tested in a previous greenhouse experiment were applied here except for pathogen control, following the same schedule. Standard agricultural practices were maintained throughout the season. PDI and lesion frequency along with growth plant parameters and yield attributes were likely measured.

2.7Effect of eco-friendly amendments on activity of defense enzyme

In apolyhouse experimenton VRI-2 variety of groundnut(as mentioned in 2.3) subsequent to inoculation with the two targetpathogens at 35 days post-sowing, plant samples were obtained at designated time points (1-, 3-, 5- and 7-days post inoculation) from each treatment.Groundnut plant tissues were immediately frozen in liquid N_2 and subsequently homogenized. A sample of1gmixed with 2 mL of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was subsequently centrifuged at 10,000 rpm for 20 min. and used for enzyme assays.

Peroxidase (PO) activity was determined by following the method of Srivastava [14]and the enzyme activity was expressed as the change in absorbance/min/g of fresh weight tissue.The polyphenol oxidase (PPO) activity was assayed as per Mayer and Harel (15). The change in absorbance at 495 nm/min/g of fresh-weight tissue is used to express PPO activity.Phenylalanine Ammonia-Lyase (PAL)was assayed following the procedure of Srivastava (14) and expressed as micromoles (µmol) of trans-cinnamic acid formed per minute per gram of fresh tissue weight (µmol min^{-1} g^{-1} fresh weight).

2. 8STATISTICAL DATA ANALYSIS

A Completely Randomized Block Design (CRBD) and Randomized Block Design (RBD) with three replicates was used for the pot culture and field experiments respectively. Statistical analysis was performed using SAS software (version 9.4, SAS Institute, Cary, NC, USA) to assess treatment effects. Following a significant analysis of variance (ANOVA),

Dunnett's multiple comparison test (DMRT) was employed to identify statistically significant differences ($p < 0.05$) between treatment means and the control. All the treatments were replicated thrice.

3.RESULTS AND DISCUSSION

3. 1 Survey on the disease incidence

Asubstantial variation in disease incidence across the surveyed locations. LLS exhibited a prevalence ranging from 8.24% to 32.84%, with the highest incidence observed in Mathur. Similarly, rust incidence varied between 7.21% and 30.78%, with Mathur again recording the most severe infection. These findings suggest that geographical factors, cultivar type, and pathogen genetics within the region may significantly influence the severity and distribution of the pathogens[16-18].The co-infection of both *P. personata* and *P. arachidis*causes severe foliar disease in all major groundnut growing areas of Tamil Nadu.

3.2 Efficacybiocontrol-based components under pot culture conditions

A significant reduction in disease incidence of LLS and rust in plants treated withseveral biocontrol based formulations was found.Among the treatments, the combination (seed and soil application) of *T.asperellum*, *P.fluorescens*, fortified lignite fly ash, and Annamalai mixture (denoted as T7) had most significantly lowered the lesion frequency of LLSand disease severity index (PDI).The same treatment also significantly reduced the disease incidence of rust and PDI of rust.Additionally, T7 resulted in a considerable increase in pod yield.The findings also suggested that strategically combined biocontrol-based formulations offer a promising approach for managing LLS and rust diseases, potentially surpassing the efficacy of individual eco-friendly components and even a standard chemical fungicide (Table 1).The microbial consortia could potentially proliferate in the soil and survive throughout the cropping season and protect the crop from harmful pathogenic infection [19,20].Earlier workers have proven that thenative isolate of bioagents could reduce the disease intensity of LLS and rust[21,22].The biocontrol agents like *Trichoderma* and *Pseudomonas* produce antibiotics, iron-chelating agents, cell wall degrading enzymes and secondary metabolites that act on pathogens tosuppress the pathogens' growth effectively [23,24]. Annamalai mixture has been proven as an effective disease suppressor and plant growth promoter[25]. Themixture contains animal excretes thatimprove the soil health and protect the plants against various diseases by promoting plant growth[13,12].

Table 1. Evaluation of biocontrol-based formulations against leaf spot and rust under pot culture condition

Treatment	Disease incidence						PDI						Yield g/plant
	Late leaf spot (No. of lesion)			Rust (No. of pustule)			Late leaf spot			Rust			
	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS	
T_1	0.98^{d}	1.14^{d}	1.32^{e}	0.64^{e}	0.89^{e}	1.27^{e}	12.9 (21.04)^{e}	13.6 (21.64)^{e}	14.1 (22.05)^{e}	10.8 (19.18)^{e}	11.2 (19.55)^{e}	13.8 (21.80)^{e}	53^{e}
T_2	1.34^{g}	1.52^{g}	2.02^{h}	1.10^{g}	1.48^{h}	1.63^{h}	15.9 (23.49)^{h}	16.1 (73.65)^{h}	16.9 (24.27)^{h}	15.2 (22.94)^{h}	16.4 (23.88)^{h}	17.7 (24.87)^{h}	50^{h}
T_3	0.63^{c}	1.02^{c}	1.24^{d}	0.49^{d}	0.71^{d}	1.09^{d}	10.3 (18.71)^{d}	11.9 (20.17)^{d}	13.6 (21.64)^{d}	7.0 (15.34)^{d}	8.3 (16.74)^{d}	11.7 (20.00)^{d}	57^{d}
T_4	1.19^{e}	1.26^{e}	1.57^{f}	0.87^{f}	1.16^{f}	1.32^{f}	14.1 (22.05)^{f}	15.3 (23.02)^{f}	15.9 (24.27)^{f}	12.5 (20.70)^{f}	13.0 (21.13)^{f}	13.9 (21.89)^{f}	53^{f}
T_5	1.27^{f}	1.44^{f}	1.63^{g}	1.05^{g}	1.39^{g}	1.52^{g}	15.0 (22.78)^{g}	15.9 (23.49)^{g}	16.3 (23.81)^{g}	14.5 (22.38)^{g}	15.3 (23.02)^{g}	16.1 (23.65)^{g}	51^{g}
T_6	0.42^{b}	0.97^{c}	1.13^{c}	0.37^{c}	0.67^{c}	1.03^{c}	9.7 (18.14)^{c}	10.2 (18.62)^{c}	12.9 (21.04)^{c}	6.2 (14.41)^{c}	7.9 (16.32)^{c}	10.8 (19.18)^{c}	59^{c}
T_7	0.31^{a}	0.62^{a}	0.98^{a}	0.18^{a}	0.31^{a}	0.60^{a}	7.3 (15.67)^{a}	8.9 (17.35)^{a}	11.3 (19.64)^{a}	5.2 (13.18)^{a}	7.1 (15.45)^{a}	9.0 (17.45)^{a}	69^{a}
T_8	0.37^{b}	0.84^{b}	1.02^{b}	0.29^{b}	0.53^{b}	0.89^{b}	8.9 (17.35)^{b}	9.8 (18.24)^{b}	12.6 (20.79)^{b}	5.9 (14.05)^{b}	7.6 (16.00)^{b}	9.9 (18.33)^{b}	63^{b}

T_9	3.11^{i}	4.56^{i}	6.05^{j}	2.17^{i}	3.63^{j}	5.22^{j}	38.3 (38.23)^{j}	41.8 (40.28)^{j}	42.1 (40.45)^{j}	35.5 (36.57)^{j}	38.7 (38.46)^{j}	41.2 (39.93)^{j}	45^{j}
T_{10}	2.45^{h}	3.26^{h}	3.40^{i}	2.03^{h}	3.25^{i}	3.47^{i}	23.8 (29.19)^{i}	28.3 (32.13)^{i}	34.9 (36.21)^{i}	19.6 (26.27)^{i}	23.7 (29.13)^{i}	30.8 (33.70)^{i}	51^{i}

* Means of three replications, Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

3. 3 Evaluation of biocontrol-based formulations components under field conditions

The results of the potculture experiments were confirmed in the field. Again, the treatment(T7) demonstrated the most significant reductions in disease incidence for both LLS and rust. Treatment T7 displayed consistently lower lesion frequency and percent disease index (PDI) of LLSand rust diseases at 50, 70, and 90DAS, respectively. Additionally, T7 resulted in the highest yield.The combined application of these biocontrol based formulations was proven to bea more effective approach for managing LLS and rust in groundnut crops compared to individual applications or a chemical fungicide control (Table 2).Combined application of bioproducts have beenknown to control diseases effectively by employing more than one mechanism of direct or indirect pathogen suppression[26,27]. Such approaches include combination of organic products with biocontrol agents[13], a combination of plant products with biocontrol agents [28,29] and a combination of fungicides with biocontrol agents [30].These bioproducts also promoted plant growth significantly and increased the yield of the crop [31].The major reason for the increased plant growth and yield parameters have been attributed to the significant induction of plant growth-promoting substances like auxin, gibberellic acid and abscisic acid induced by thebioproducts [32,33].The lignite fly ash used as carrier material contains a trace amount of minor nutrients like Ca, K, and Si[34]. These minor nutrients trigger the plant defense response and protect the plants [35].

Table 2. Evaluation of various eco–friendly components against *Phaeoisariopsis personata* and *Puccinia arachidis* under field condition

Treatment	Disease incidence						PDI						Yield kg/ha
	Late leaf spot (No. of lesion)			Rust (No. of pustule)			Late leaf spot			Rust			
	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS	50 DAS	70 DAS	90 DAS	
T_1	0.38^{c}	0.54^{c}	0.72^{c}	0.34^{c}	0.38^{c}	0.43^{c}	10.09 (18.52)^{e}	10.79 (19.17)^{e}	10.97 (19.34)^{e}	6.80 (15.11)^{e}	7.14 (15.49)^{e}	8.83 (17.28)^{e}	1893^{e}
T_2	0.48^{d}	0.61^{d}	0.79^{d}	0.42^{d}	0.48^{d}	0.53^{d}	11.76 (20.05)^{h}	12.13 (20.38)^{h}	12.69 (20.86)^{h}	8.87 (17.32)^{h}	9.24 (17.67)^{h}	10.19 (18.61)^{h}	1828^{h}
T_3	0.35^{b}	0.50^{c}	0.69^{b}	0.29^{c}	0.31^{b}	0.39^{b}	9.14 (17.59)^{d}	9.39 (17.84)^{d}	10.51 (18.91)^{d}	5.93 (14.09)^{d}	6.94 (15.27)^{d}	8.02 (16.45)^{d}	1897^{d}
T_4	0.41^{c}	0.56^{d}	0.75^{c}	0.37^{d}	0.42^{c}	0.47^{c}	10.51 (18.91)^{f}	10.93 (19.30)^{f}	11.39 (19.72)^{f}	7.46 (15.85)^{f}	7.91 (16.33)^{f}	9.09 (17.54)^{f}	1868^{f}
T_5	0.46^{d}	0.59^{d}	0.77^{c}	0.40^{d}	0.45^{d}	0.49^{d}	11.19 (19.54)^{g}	11.78 (20.07)^{g}	12.04 (20.30)^{g}	8.51 (16.96)^{g}	8.89 (17.34)^{g}	9.71 (18.15)^{g}	1843^{g}
T_6	0.32^{b}	0.47^{b}	0.66^{b}	0.23^{b}	0.30^{b}	0.37^{b}	8.42 (16.86)^{c}	8.96 (17.41)^{c}	10.39 (18.80)^{c}	5.02 (12.94)^{c}	6.27 (14.50)^{c}	7.98 (16.40)^{c}	1938^{c}

T_7	0.22[a]	0.38[a]	0.51[a]	0.12[a]	0.24[a]	0.31[a]	7.20 (15.56)[a]	8.14 (16.57)[a]	9.50 (17.95)[a]	3.92 (11.41)[a]	5.67 (13.77)[a]	6.81 (15.12)[a]	2060[a]
T_8	0.27[a]	0.44[b]	0.56[a]	0.19[b]	0.27[a]	0.33[a]	8.32 (16.76)[b]	8.89 (17.34)[b]	10.07 (18.50)[b]	4.19 (11.81)[b]	6.05 (14.23)[b]	7.37 (15.75)[b]	1984[b]
T_9	1.36[e]	1.51[e]	1.68[e]	1.31[e]	1.33[e]	1.38[e]	14.46 (22.34)[i]	16.89 (24.26)[i]	18.45 (25.43)[i]	10.67 (19.06)[i]	12.02 (20.28)[i]	18.48 (25.46)[i]	1739[i]

* Means of three replications, Values in the column followed by same letters not differ significantly by DMRT (p=0.05).

(For treatment descriptions, please refer to the foot note in the Table 1. T10 was not present in field experiment).

3. 4 Defense enzyme activity in groundnut plants against *P. personata*and *P. arachidis*

Combined application of bioproducts (treatment T7) resulted in the most significant induction of activities of the defense enzymes,PO, PPOand PAL. This peak induction occurred 5 days post-challenge inoculation with the respective pathogens. Interestingly, plants solely inoculated with the pathogens also exhibited elevated levels of defense enzymes compared to the healthy control group up to the fifth day (Fig.1). This stimulation of defense enzymes suggests that the combined application of these biocontrol components possibly are involved in systemic resistance in groundnut plants, potentially improving their tolerance against these fungal diseases [36].Peroxidases are a diverse group of enzymes that detoxify reactive oxygen species (ROS) produced by pathogens [37].These ROS are highly reactive and can damage cellular components [38]. By detoxifying ROS, peroxidases prevent oxidative damage to proteins, lipids, and nucleic acids, maintaining cellular integrity and promoting plant survival[39]. PPO catalyzes the oxidation of phenolic compounds, leading to the formation of quinones. These quinones disrupt pathogen membranes and inactivates their enzymes, directly inhibiting infection(39).

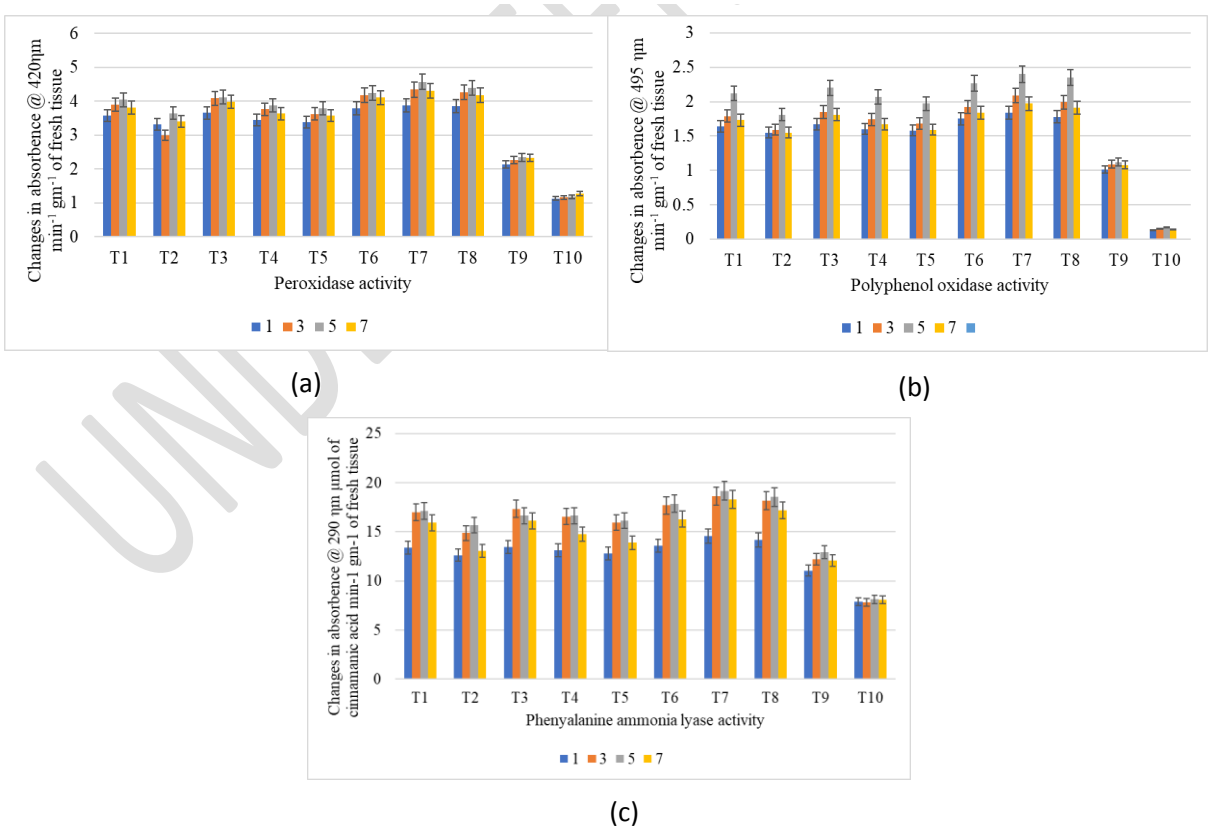

Fig. 1. Induction of defence enzymes by eco-friendly components in groundnut plants against *Phaeoisariopsispersonata* and *Pucciniaarachidis*.(a) Peroxidase, (b) Polyphenol oxidase, (c) Phenylalanine ammonia-lyase.(For treatment descriptions, please refer to the foot note in the Table 1).

4. CONCLUSION

Surveys of Late Leaf Spot(LLS) and rust in groundnut crops across the Cuddalore district, Tamil Nadu, India revealed significant geographical variation in disease incidence, with Mathur exhibiting the highest infection rates for both diseases. A combination of *Trichodermaasperellum*, *Pseudomonasfluorescens*, fortified lignite fly ash and Annamalai mixture most significantly reduced the disease incidence for both LLS and rust and increased groundnut yieldunder both pot culture and field conditions. Induction of defense enzyme activity in groundnut plants treated with these biocontrol-based components suggests a potential mechanism for disease resistance.

6. REFERENCE

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