Original Research Article

Efficacy of Combined Eco-Friendly Formulation for Managing Late Leaf Spot and Rust Diseases and Enhancing Yield in Groundnut

ABSTRACT

This study investigated the efficacy of various eco-friendly components against 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 eco-friendly formulations compared 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 (0.38, 0.72 and 1.08) and Percent Disease Index (PDI)(8.0, 9.4 and 12.5) for both diseases at (50, 70 and 90 DAS) respectively, compared to other treatments. The combined eco-friendly treatment also significantly increased the pod yield (67g/plant). Furthermore, the same treatment increased the induction of defence enzymes peroxidase (2.22fold), polyphenol oxidase (1.28 fold) and phenylalanine ammonia-lyase (6.25 fold). While Carbendazim, a chemical fungicide, effectively reduced disease incidence, it was surpassed by the combined eco-friendly treatment in terms of both disease control and yield.These findings suggest that a combination of eco-friendly formulations holds promise as a sustainable approach for managing LLS and rust diseases in groundnut cultivation. This method offers potential benefits over chemical fungicides, including reduced environmental impact and potentially higher crop yields.

Keywords: Defence enzyme, survey, fortified lignite fly ash,Annamalai mixture, groundnut

1. INTRODUCTION

Groundnut (*Arachishypogaea* L.), belonging to the Fabaceae family, is a globally important legume prized for its nutritional and agricultural contributions. Often hailed as the "king of oilseeds," Globally, India holds a dominant position, holding the largest cultivation areaand 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). Groundnut offers a compelling nutritional profile, rich in edible oil and vegetable protein[1]. Beyond its direct benefits, this legume fosters soil health through symbiotic nitrogen fixation with Rhizobia bacteria, promoting sustainable agricultural practices.

Despite its economic and nutritional significance, 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[2,3]. Among these, LLSdisease caused by *Phaeoisariopsispersonata* 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[4]. In India, these diseases have been documented to cause yield losses exceeding 70%, significantly impacting agricultural productivity[5]. Leaf rust alone could cause yield reduction reaching up to 65%, especially in areas with high rainfall [6]. 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. Nevertheless, their indiscriminate use raises

concerns about environmental and human health risks[2].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[7,8].The emergence of eco-friendly alternatives presents a promising solution for sustainable groundnut production[9]. 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 [10]. Additionally, some antagonists have the potential to induce systemic resistance within the plant itself[11]. These mechanisms offer a multifaceted approach to disease control, potentially mitigating the need for harmful chemical fungicides.

This research delves into the potential of eco-friendly components, particularly antagonist microorganisms, as a strategy to enhance groundnut's defense mechanisms against the detrimental fungal pathogens, *P.personata* and *P.arachidis*. By exploring this approach, we aimed to the development of sustainable and effective disease management practices for groundnut cultivation.

2. MATERIALS AND METHOD

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

This study employed a field survey approach 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.

2.3Artificial Inoculation Method

Groundnut seeds (cv. VRI 2) were grown in pots (20 cmdia, 5 plants/pot) for 30 days within a polyhouse environment. 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 suspension containing both *P. personata* and *P. arachidis*. 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 12 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*. (12).

2. 3. 1Assessment of disease severity

Quantification of lesion incidence in response to various treatments for combined LLS and rust disease [4].

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

Disease scoring was done with modified 9-point (1-9) scale [12].

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

2. 4 Annamalai mixture preparation

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. The

resulting organic amendment was subsequently diluted to 20%, 40%, 60%, and 80% (v/v) using distilled water at the time of application[13].

2. 5 Lignite fly ash fortification

T.asperellum and *P. fluorescens,* the bio-inoculants were cultivated in a 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. Followed by 400 ml of each bacterial suspension, containing approximately 8×10^9 CFU/mL, were separately added to 1000 g of sterilized LFA-CMC carrier. 20 ml of molasses were then incorporated into the mixture, followed by thorough mixing. The fortified LFA was shade-dried for 2 hours 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[14].

2. 6Efficacy of eco-friendly products against LLS and rust in groundnut under pot culture condition

A study was carried out under pot culture conditions to assess the effect of various eco-friendly components against LLS and rust in groundnut. 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. Eco-friendly 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. 7 Efficacy of eco-friendly amendments against *P.personata* and *P.arachidis* in field-grown groundnut

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 same eco-friendly 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. 8 Effect of eco-friendly amendments on defenceenzyme induction in groundnut against *P. personata* and*P. arachidis*

2. 8. 1 Sample Collection

A polyhouse experiment was employed to evaluate the efficacy of various eco-friendly component formulations in inducing defense enzyme activity within VRI-2 variety against *P. personata* and *P.arachidis*.Subsequent inoculation with the respective pathogens at 35 days

post-sowing, plant samples were obtained at designated time points (1-, 3-, 5- and 7-days post inoculation) from each treatment.

2. 8. 2 Enzyme extraction

Groundnut plant tissues were immediately frozen in liquid N_2 and subsequently homogenized. A 1g aliquot of the powdered sample was homogenized 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. The activity of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) were assayed in the resultant supernatant.

2. 8. 3 Enzymaticassay

2. 8. 3. 1 Peroxidase (PO)

PO activity was determined by following the method of Srivastava [15]. The reaction mixture contained 1.5 mL of 0.05 M pyrogallol, 0.5 mL of enzyme extract, and 0.5 mL of 1% hydrogen peroxide (H_2 O_2) in a final volume of 2.5 mL. The mixture was incubated at 28±1°C. Absorbance change at 420 nm was monitored every 30 secfor a total duration of 3 min. Aboiled enzyme preparationservedas a negative control. The enzyme activity was expressed as the change in absorbance/min/g of fresh weight tissue.

2. 8. 3. 2 Polyphenol Oxidase (PPO)

This assay measures PPO activity through the oxidation of catechol at 495 ηm. The reaction mixture comprises 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5), 200 µL of enzyme extract, and 200 µL of 0.01 M catechol. The change in absorbance at 495 nm/min/g of fresh-weight tissue is used to express PPO activity [16].

2. 8. 3. 3Phenylalanine Ammonia-Lyase (PAL)

In a cuvette, 100 µL of enzyme solution was combined with 500 µL of 50 mM Tris-HCl buffer (pH 8.8) and 600 µL of 1 mM L-phenylalanine substrate solution. This reaction mixture was incubated for 60 minutes at 32± 2ºC to allow enzymatic conversion of the substrate. The reaction was then quenched by the addition of 2 N HCl, stopping further enzyme activity. To extract the product, trans-cinnamic acid, 1.5 mL of toluene was added, and the mixture was vigorously vortexed for 30 seconds to facilitate the transfer of the product into the organic phase. The mixture was then centrifuged at 1000 rpm for 5 minutes to achieve phase separation. The upper toluene layer, containing the extracted trans-cinnamic acid, was carefully transferred and its absorbance was measured at 290 ηm using a spectrophotometer. A blank cuvette containing only toluene served as the reference for background subtraction. A standard curve for trans-cinnamic acid was previously established using known concentrations of the product in toluene. The enzyme activity was then calculated 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)[15].

2. 9 STATISTICAL DATA ANALYSIS

A randomized block design (RBD) with three replicates was used for the pot culture and field experiments. 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

A spatial survey was conducted in various locations within the Cuddalore district, TamilNadu, to assess the prevalence of LLS and rust diseases in groundnut crops. The investigation revealed a substantial 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 influencing the severity and distribution of the pathogens[17-19].The co-infection of both *P. personata* and *P. arachidis* are causes severe foliar disease in all major groundnut growing areas of Tamil Nadu.

3.2 Evaluation of various eco–friendly components against *P. personata* and*P. arachidis* isolates under pot culture conditions

The study investigated the effectiveness of eco-friendly formulations compared to a control group. Results demonstrated a significant reduction in disease incidence of LLS and rust for several eco-friendly formulations compared to the control.Among the treatments, the combination (seed and soil application) of *T.asperellum*, *P.fluorescens*, fortified lignite fly ash, and Annamalai mixture (denoted as T7) exhibited the most pronounced disease reduction. T7 significantly lowered the lesion frequency of LLS (0.38, 0.72 and 1.08)and disease severity index (PDI)(8.0, 9.4 and 12.5) at various observation points (50, 75 and 90 DAS) respectively.The same treatment also significantly reduced the disease incidence of rust which recorded 0.24, 0.51, 0.83 lesion frequency and 5.9, 7.6, 9.8 PDI of rust at 50, 70 and 90 DAS respectively.Additionally, T7 resulted in a considerable increase in pod yield (67 g/plant).The performance of T7 was followed by the application of carbendazim (chemical fungicide) which also displayed a significant reduction inLLS (0.47, 0.94 and 1.12 lesion frequency and 9.2, 10.8 and 13.7 PDI) andrust (0.49, 0.73, 0.99 lesion frequency and 6.9, 8.1, 10.7 PDI) at 50, 70 and 90 DAS respectively, compared to the control. However, the combined eco-friendly formulation (T7) demonstrated superior disease control and yield improvement compared to the chemical treatment (carbendazim). Overall, the findings suggest that strategically combined eco-friendly 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. and Fig.1). Being a combination of biocontrol agents and eco-friendly amendments is an added advantage in that the microbial consortia could potentially proliferate in the soil and survive throughout the cropping season and protect the crop from harmful pathogenic infection [20,21]. Thenative isolate of bioagents performs well ultimately reducing the disease intensity of LLS and rust[22,23].During the tripartite interaction, the biocontrol agents like *Trichoderma* and *Pseudomonas* produce certain kind of antibiotics, iron-chelating agents, cell wall degrading enzymes and secondary metabolites that acts on pathogensleads to suppressing the pathogens' growth effectively [24,25]. Annamalai mixture has been proven as an effective disease suppressor and plant growth promoter. This mixture contains animal excretes are improve the soil health and protect the plants against various diseases by promoting plant growth[14,13].

Table 1. Evaluation of various eco–friendly components against *Phaeoisariopsispersonata* and *Puccinia arachidis* 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)

Fig. 1. Pot culture study of eco-friendly components against *Phaeoisariopsispersonata* and *Puccinia arachidis*

3. 3 Evaluation of various eco–friendly components against *P. personata* and *P. arachidis* isolates under field conditions

The experiment investigated the impact of these eco-friendly formulations on disease incidence and yield compared to a control. The aforesaid 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 LLS(0.22, 0.38, 0.51 and 7.20, 8.14, 9.50) and rust (0.12, 0.24, 0.41 and 3.92, 5.67, 6.81) diseases at 50, 70, and 90DAS, respectively. Additionally, T7 resulted in the highest yield (2060 kg/ha).Carbendazim application (T8) served as a positive control and exhibited a lower diseaseincidence of late leaf spot (0.27, 0.44, 0.56 lesion frequency and 8.32, 8.89, 10.07 PDI) and rust (0.19, 0.27, 0.33 lesion frequency and 4.19, 6.05, 7.37 PDI) at 50, 70 and 90 DAS respectively, compared to the control but was surpassed by the combined eco-friendly formulation treatment (T7). Treatment T8 displayed a moderate reduction ofLLS and rust

along with a yield of 1984 kg/ha.The study suggests that the combined application of these eco-friendly formulations offers a more effective and potentially more sustainable approach for managing LLS and rust in groundnut crops compared to individual applications or a chemical fungicide control (Table 2& Fig 2).Overall, the combined application of bioproducts is known to control diseases effectively by employing more than one mechanism of direct or indirect pathogen suppression[26,27]. This approach has been evaluated against various diseases with a combination of a variety of bioproducts including a combination of biocontrol agents[27], a combination of organic products with biocontrol agents[14], a combination of plant products with biocontrol agents [28,29] and a combination of fungicides with biocontrol agents [30].These bioproducts also promote plant growth significantly and increase the yield of the crop [31-33].The major reason for the increased plant growth and yield parameters might be due to the significant induction of plant growth-promoting substances like auxin, gibberellic acid and abscisic acid induced by thebioproducts [34,35].The lignite fly ash used as carrier material contains a trace amount of minor nutrients like Ca, K, and Si[36-38]. These minor nutrients trigger the plant defence response and protect the plants [39-41].

Table 2. Evaluation of various eco–friendly components against *Phaeoisariopsispersonata* 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)

Fig. 2. Field study of eco-friendly components against *Phaeoisariopsispersonata* and *Puccinia arachidis*

3. 4 Defence enzyme activity in groundnut plants to eco-friendly amendments against *P. personata*and rust *P. arachidis*

Plants rely on a robust enzymatic defense system to combat various threats, including pathogens, herbivores, and abiotic stresses. Pot culture experiments investigated the influence of various eco-friendly components on defense enzyme activity in groundnut plants challenged with *P.personata*and*P.arachidis*. The eco-friendly bio products included *T.asperellum*, *P. fluorescens*, fortified lignite fly ash and Annamalai mixture applied as mentioned previously. Combined application of bioproducts (treatment T7) resulted in the most significant induction of defense enzyme activities PO (2.22-fold), PPO (1.28-fold) and PAL (6.25-fold). This peak induction occurred 5 days post-challenge inoculation with the respective pathogens (Fig. 2). Treatment T8 also displayed substantial enzyme activity increases, although to a lesser extent than T7.Overall, enzyme activity declined following the fifth day of pathogen inoculation across all treatments. 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. 3). This stimulation of defense enzymes suggests that the combined application of these eco-friendly components triggers systemic resistance in groundnut plants, potentially improving their tolerance against these fungal diseases [42]. These results are supported by previous studies demonstrating the induction of defense enzymes in plants treated with biocontrol agents and other eco-friendly products [43,44].Peroxidases are a diverse group of enzymes that detoxify reactive oxygen species (ROS) produced by pathogens [45,46].These ROS, like hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), are highly reactive and can damage cellular components [47]. By detoxifying ROS, peroxidases prevent oxidative damage to proteins, lipids, and nucleic acids, maintaining cellular integrity and promoting plant survival[48]. In addition to ROS detoxification, plants employ Polyphenol Oxidase (PPO) to fortify their defenses. PPO catalyzes the oxidation of phenolic compounds, leading to the formation of quinones. These quinones play a multifaceted role in contributing to cell wall stiffening, triggering phytoalexin synthesis [49]. This disrupts pathogen membranes and inactivates their enzymes, directly inhibiting infection. Additionally, these bioproducts trigger the induced systemic resistance (ISR) in plants against pathogenic organisms[50]. Overall, these findings are

consistent with previous reports suggesting that various modes of action of these eco-friendly components, including rhizosphere colonization [51], antibiotic production[52], and induction of systemic resistance[50], contribute to their potential as biocontrol agents for managing pests and diseases in crop plants. The enhanced defense enzyme activity observed in plants treated with the combination of eco-friendly components suggests their role in strengthening the plant's defense mechanisms against pathogen attack.

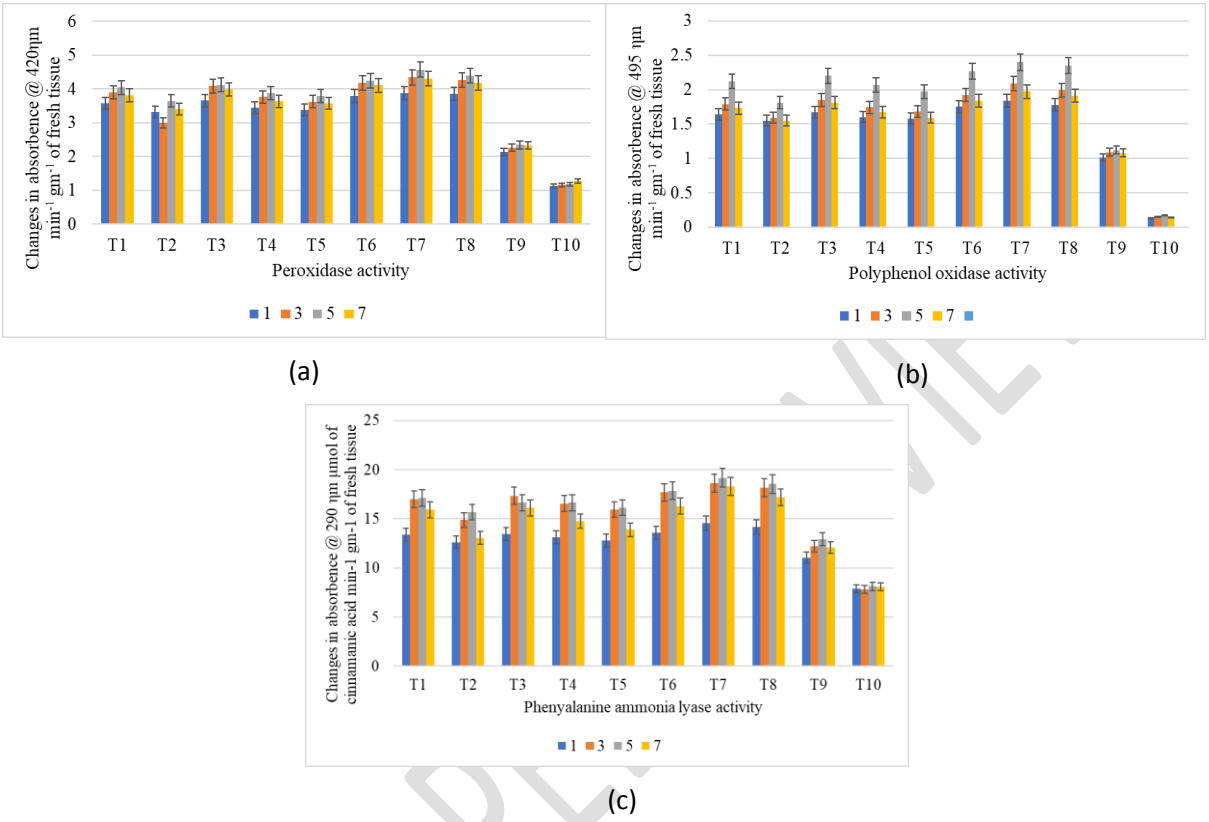

Fig. 3. Induction of defence enzymes by eco-friendly components in groundnut plants against *Phaeoisariopsispersonata* and *Pucciniaarachidis.* (a) Peroxidase, (b) Polyphenol oxidase, (c) Phenylalanine ammonia-lyase.T_1 – Talc formulation of *T. asperellum*as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS, T_2 – Talc formulation of *P. fluorescens*as seed treatment @ 10g/kg of seed, soil application @ 10 kg/ha at 25 and 45 DAS, T_3 – T_1 +T_2, T_4 – Application of fortified lignite fly ash as seed treatment @ 10g/kg of seed, soil application @ 40 kg/ha at 25 DAS, T_5 – Application of Annamalai mixture as seed @ 10 ml/kg of seed, foliar spray @ 20 lit/ha at 25 and 45 DAS,T_6 – T_3 + T_4, T_7– T_6 + T_5, T_8 – Carbendazim seed treatment @ 2g/kg, foliar application @ 0.1 g/lit at 25 and 45 DAS, T_9 – Inoculated control and T_{10} – Healthy control.

4. CONCLUSION

This study investigated the prevalence of LLS and rust rot diseases in groundnut crops across the Cuddalore district, Tamil Nadu, India. The findings revealed significant geographical variation in disease incidence, with Mathur exhibiting the highest infection rates for both diseases. This suggests that location-specific factors likely play a role in disease distribution and severity.Furthermore, the research explored the efficacy of various eco-friendly components in managing these fungal diseases. A combination of *T. asperellum*, *P.fluorescens*, fortified lignite fly ash and Annamalai mixture demonstrated the most significant reduction in disease incidence for both LLS and rust rot under both pot culture and field conditions. This eco-friendly approach also resulted in increased groundnut yield

compared to control groups. Additionally, the study observed induction of defense enzyme activity in groundnut plants treated with eco-friendly components, suggesting a potential mechanism for disease resistance.These findings highlight the potential of eco-friendly formulations for managing LLS and rust rot in groundnut crops, offering a sustainable alternative to chemical fungicides.

8. CONSENT

All the authors approved the manuscript at the time of submission

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