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Combined effect of cauliflower intercropping and *Pseudomonas fluorescens* seed biopriming on the suppression of fusarium wilt in okra

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Use of chemical pesticides and fertilizers impart negative impact on human health and environment. There is a need to develop alternate strategies for the management of phyto-pathogens. Intercropping offers a sustainable solution for plant disease management by modulating microbial population in rhizosphere and reducing pathogen load. In this study, we examined the protective impact of cauliflower (*Brassica oleracea*) and *Pseudomonas fluorescens* (Pfl) as seed priming agent against *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) induced wilt in okra (*Abelmoschus esculentus*). Intercropping cauliflower with okra resulted in reduction of disease incidence and severity (44% and 67%, respectively) compared to okra cultivated with okra. Okra plants intercropped with cauliflower maintained higher growth in terms of root and shoot dry weight (10.94% and 7.50%, respectively) as well as root and shoot length (2.2% and 33.7%, respectively) compared to okra grown in combination with okra under fusarium wilt stress. Maximum content of leaf nitrogen, phosphorus and potassium (5.52, 1.14, and 1.98%, respectively) was recorded in *P. fluorescens* primed okra co-cultivated with cauliflower under wilt stress. Root exudates extracted from okra intercropped with cauliflower reduced FOV microconidia germination by 23.58% compared to exudates from okra cultivated with okra. Higher activity of peroxidase, glucanase and polyphenol oxidase (3.54, 5.01 and 3.01 Units / min/g FW, respectively) was recorded in okra intercropped with cauliflower and inoculated with *P. fluorescens* compared to unprimed okra intercropped with cauliflower (2.92, 4.56 and 2.27 Units / min/g FW, respectively) under FOV infection. In the presence of fusarium infection, okra companioned with cauliflower and primed with *P. fluorescens* successfully maintained higher photosynthetic activity and leaf chlorophyll content (3.93 mg/g FW) compared to okra cultivated with cauliflower in the absence of *P. fluorescens* (3.78 mg/g FW). Overall, our findings revealed that the synergy of cauliflower intercropping and priming okra with *P. fluorescens* not only reduced the impact of fusarium induced infection but also enhanced growth parameters of okra.

Keywords Companion cropping, *Pseudomonas fluorescens*, Biological control, *Fusarium oxysporum* f. sp *vasinfectum*

Okra is a vegetable crop of prime importance in warm, subtropical and tropical regions of Asia, Africa, Europe and America[1]. With a global production of over 6 million tons per year, okra is a highly valuable commodity[2]. Okra contain large amounts of carbohydrates, vitamins, calcium and proteins[3]. However, okra cultivation is currently facing several biotic and abiotic challenges[4], including diseases induced by fungal pathogens such as powdery mildew, Cercospora leaf spot, pod rot and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) induced wilt[5,6]. Among these pathogenic fungi, fusarium wilt of okra (*Fusarium oxysporum* f. sp. *vasinfectum*; FOV) was documented to induct serious reduction of per acre yield with infection rate ranging from 18 to 78%[7],

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amounting of over $10 million[8,9]. Globally, economic losses caused by fusarium wilt of okra range from 430 to 3000 $ per hectare[10,11].

Fusarium wilt, a seed-borne fungus that can persist in soil as saprophyte for extended periods, making its management difficult[12,13]. Traditionally, fusarium induced infection in okra restricted through the use of chemical fungicides such as Topspin, Benomyl, Carbendazim and Difenoconazole. However, application of synthetic fungicides lead to lethal effects on environment and human health along with evolution of fungicide resistant strains[14]. Genetic resistance in okra against FOV can be considered as an alternative to chemical control[15]. But genes for resistance in okra provide only moderate level of protection. Currently no cultivar of okra is known to be immune against FOV[16].

Moreover, as a consequence of mono-cropping, build-up of pathogen load in the soil resulted into increased disease index with each growing season[17].To address these challenges, there is urgent need to employ environment friendly materials as well as alternative sustainable strategies that employ diverse mechanisms of actions to effectively supress the proliferation of pathogens[18]. In order to make the soil conditions less favorable for the pathogen, intercropping can serve as sustainable disease management tool[19]. Intercropping not only enhance soil fertility, crop yield but also play a crucial role in mitigation of soil borne pathogens. For example, co-cultivation of wheat with faba bean resulted into reduced fusarium wilt incidence[20]. Likewise, Zhang et al.[21] reported a drop in disease index of Verticillium wilt in cotton by interplanting with garlic. Furthermore, cultivation with anise reduced severity of root rot and damping off as well as enhanced grain yield in lentils due to a decrease in population of *Fusarium solani* and *Rhizoctonia solani*, respectively[22].

Different mechanisms operate behind suppression of soil borne pathogens due to intercropping. Change in soil microclimate due to non-host intercrop limits survival of pathogens[23]. Intercropping enhance the numbers of antagonistics in soil[24], as well as enhance lignification and upregulating of defense related genes[25]. Intercropped plants may trigger induced resistance in the main crop. In this way, main crops become immune against pathogens[26]. Intercropped plants release various secondary metabolites in the form of root exudates. Root exudates modify soil properties to make it suppressive against soil borne pathogens[27]. These exudates or volatile compounds lead to suppression of soil pathogens and nematodes[28,29].

The plant growth promoting rhizobacteria (PGPR) such as Pseudomonas spp., Bacillus sp., and Azospirillum spp. successfully colonize rhizosphere and positively influence plant growth[30]. Both direct (Siderophore production, phytohormones secretion, N_2-fixation, and nutrient solubilization) as well as in-direct (induction of systemic resistance, production of cell wall degrading enzymes and synthesis of antibiotics) mechanisms are employed by PGPR to enhance plant growth and suppression of pathogens in the rhizosphere[31]. In a field experiment performed by Selvaraj et al.[32], 59.9% reduction in banana fusarium wilt incidence was observed along with 45% increase in yield due to application of *P. fluorescens*. PGPR rely on exudations from pant roots to fulfil their metabolic needs. In return, PGPR secrete valuable compounds useful for plants[33]. This interaction results into a give and take process between plant and PGPR.

Cauliflower root exudates contain sulfur containing compounds known as glucosinolates[34]. Glucosinolates are enzymatically converted to antifungal compounds (isothiocyanates) upon fungal infection[35]. Isothiocyanates cause cell membrane disruption, depolarize the mitochondrial membrane, decrease the oxygen consumption rate and increase intracellular reactive oxygen species accumulation[36,37]. Soil amendment with residues of cauliflower reduced the incidence of *Verticillium dahliae* in artichoke due to emission of toxic volatile compounds from cauliflower residue[38]. Shavnam and Raj[39] reported mitigation of *Fusarium oxysporum* induced infection in pepper due to bio fumigation of cauliflower waste mixed in soil. Earlier studies focused on interactive effect cropping partner and different beneficial microbes for sustainable management of fungal diseases in legumes and grain crops[40–44]. However, most of the research has explored the single factor intervention i.e. either beneficial microbes or intercropping systems for mitigation of fungal infections associated with vegetables[45–47]. Very few studies had examined such interplay between companion-cropping systems and beneficial microbes for sustainable management of diseases induced by fungi in vegetables[48,49]. To fill the research gap, our study examined the combined effect of cauliflower and PGPR on mitigation of FOV induced wilt in okra.

Therefore, the key objectives of the study were to determine the interactive effect of cauliflower as an intercropping partner and PGPR for the alleviation of okra wilt, as well as investigating the inhibitory potential of okra root exudates on microconidia germination of *Fusarium oxysporum* f. sp. *vasinfectum*. The present study also included the analysis of physiological defense responses in okra co-cultivated with cauliflower and primed with PGPR. The outcome of the current work will help in our understanding of sustainable disease management of okra wilt in eco-friendly manner.

Results

Agronomic analysis

Root and shoot dry weight

There was a highly significant ($p < 0.01$) two-way interactive effect was recorded between Pfl and cauliflower (Table 1). Overall, synergistic effect of cauliflower intercropping and *P. fluorescens* biopriming significantly improved root biomass in okra. Root weight was drastically reduced across all the FOV infected treatments, however, the minimum reduction (13.5%) in root dry weight was observed in intercropped okra plants primed with *P. fluorescens* (OK^{Pfl}+CF+FOV) as compared to their un-inoculated respective control (OK^{Pfl}+CF-FOV) (Fig. 1a). Maximum root dry weight (0.33 g) was recorded in *P. fluorescens* primed okra plants intercropped with cauliflower in the absence of pathogen (-FOV). While, minimum dry weight (0.11 g) was recorded in the unprimed okra grown in association with okra under pathogen (+FOV) stress. Combined effect of intercropping and *P. fluorescens* resulted in significant reduction in okra shoot dry weight loss under stress conditions (Fig. 1b). Fusarium infection caused maximum (25%) drop in shoot dry weight in unprimed okra grown with okra (OK + OK+FOV) compared to their non-infected counterpart (OK + OK-FOV). Bio-protective

	Root Length (cm)		Shoot Length (cm)		Root dry weight (g)		Shoot dry weight (g)		Total chlorophyll		Peroxidase		Glucanase		PPO		*N*		*P*		K		Mg	
Source of variation	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Main effects																								
FOV	253.97	***	113.55	***	378.78	***	369.92	***	305.83	***	83.19	***	190.04	***	18.19	***	1.86	ns	2.43	ns	0.36	ns	2.06	ns
Pfl	0.00	ns	103.07	***	292.52	***	747.56	***	0.11	ns	18.06	***	19.85	***	43.80	***	24.16	***	21.88	***	3.40	ns	5.81	*
CF	107.39	***	13.84	***	30.30	***	123.77	***	61.92	***	5.59	*	15.25	***	14.27	***	54.03	***	120.95	***	23.65	***	3.42	ns
2-way interactions																								
FOV × Pfl	20.42	***	2.11	ns	0.04	ns	0.89	ns	6.86	*	0.70	ns	0.01	ns	0.90	ns	0.08	ns	0.25	ns	0.18	ns	0.03	ns
FOV × CF	11.81	**	13.16	***	15.46	***	0.89	ns	0.34	ns	0.16	ns	7.40	*	4.71	*	0.03	ns	3.50	ns	0.31	ns	0.03	ns
Pfl× CF	1.62	ns	3.65	ns	44.68	***	81.92	***	6.12	*	0.58	ns	0.01	ns	6.06	*	1.03	ns	0.00	ns	0.19	ns	0.67	ns
3-way interaction																								
FOV × Pfl × CF	3.69	ns	5.72	*	0.00	ns	0.57	ns	2.64	ns	2.34	ns	2.91	ns	5.56	*	0.48	ns	0.00	ns	0.26	ns	0.40	ns

Table 1. Three-way analysis of variance (ANOVA) for okra agronomic parameters, chlorophyll, enzymes and nutrient contents using *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), *Pseudomonas fluorescens* (Pfl) and cauliflower (CF) as intercropping partner. Asterisks indicate statistical significance levels: *** significant at $p < 0.001$; ** significant at $p < 0.01$; * significant at $p < 0.05$; *ns*: non-significant ($p \geq 0.05$).

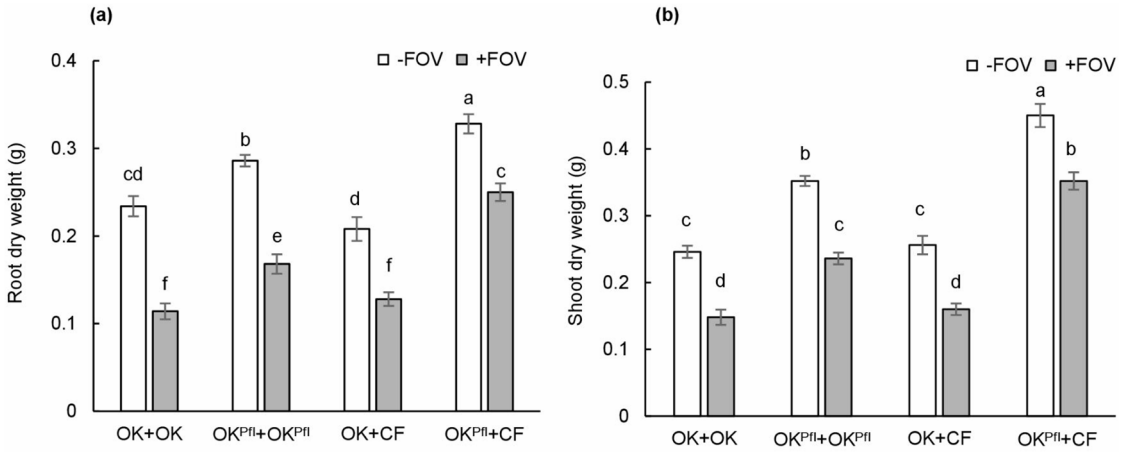


Fig. 1. Impact of intercropping partner and *Pseudomonas fluorescens* on inoculated (+FOV) and uninoculated (–FOV) okra root and shoot dry weight. **(a)** Root dry weight and **(b)** shoot dry weight of okra cultivated with okra (OK + OK), okra cultivated with okra after priming with *P. fluorescens* (OK^{Pfl}+OK), okra cultivated with cauliflower as intercropping partner (OK + CF) and *P. fluorescens* treated okra grown in association with cauliflower as intercropping partner (OK^{Pfl}+CF). Each bar represents mean ± SE, $n = 5$, recorded 58 days after transplantation. Bars with different letters at top represent significant difference as per Tukey's HSD test ($P \leq 0.05$).

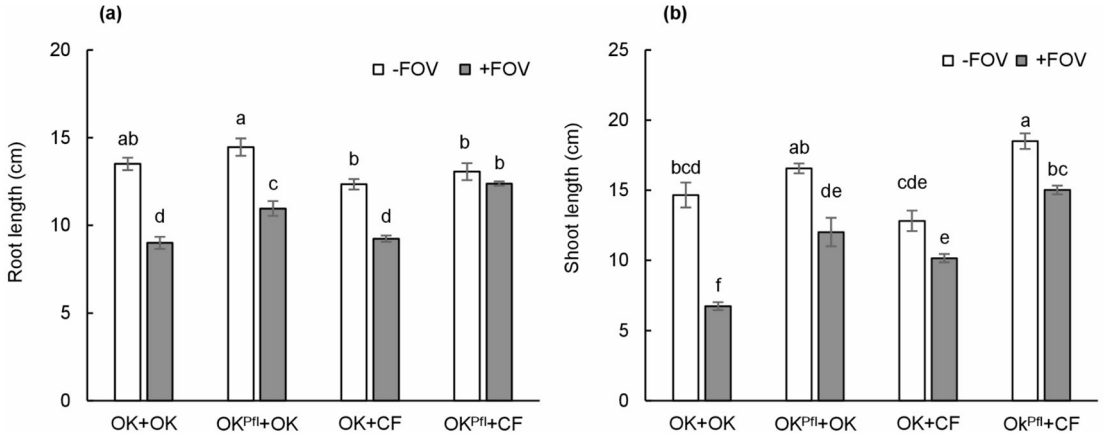


Fig. 2. Impact of intercropping partner and *Pseudomonas fluorescens* on inoculated (+FOV) and uninoculated (–FOV) okra root and shoot length. **(a)** Root length and **(b)** shoot length of okra cultivated with okra (OK + OK), okra cultivated with okra after priming with *P. fluorescens* (OK^{Pfl}+OK), okra cultivated with cauliflower as intercropping partner (OK + CF) and *P. fluorescens* treated okra grown in association with cauliflower as intercropping partner (OK^{Pfl}+CF). Each bar represents mean ± SE, $n = 5$, recorded 58 days after transplantation. Bars with different letters at top represent significant difference as per Tukey's HSD test ($P \leq 0.05$).

potential of *P. fluorescens* reduced the weight loss to 20% when okra was co-cultivated with okra (OK^{Pfl}+OK^{Pfl} + FOV). Allelopathic effect of cauliflower as companion crop further improved antifungal efficacy of *P. fluorescens* (OK^{Pfl}+CF + FOV) by minimizing okra shoot biomass reduction to just 12%.

Okra root and shoot length

The okra root length was significantly influenced by the two-way interactive effect FOV × Pfl and FOV × CF. Fusarium wilt presence resulted into a negative impact on both root and shoot length (Table 1). No significant differences were observed in root length of un-inoculated (-FOV) okra plants grown in companionship with cauliflower (OK + CF) either in the presence or absence of *P. fluorescens* (Fig. 2a). However, 21.78% higher root length was recorded when *P. fluorescens* primed okra grown in association with okra (OK^{Pfl}+OK) as compared to OK + OK combination under disease stress. Fusarium infection (+FOV) significantly reduced root length in all the treatments except in OK^{Pfl}+CF combination. Fusarium induced root length reduction was minimum (4.76%) in *P. fluorescens* primed okra grown in association with okra (OK^{Pfl}+CF).

Treatment		*N*% ± SD	*P* % ± SD	K % ± SD	Mg % ± SD
OK + OK	-FOV	3.81 ± 0.39^{d}	0.31 ± 0.10^{d}	1.28 ± 0.19^{b}	0.65 ± 0.12^{bc}
OK^{Pfl} + OK		4.20 ± 0.58^{cd}	0.51 ± 0.14^{cd}	1.41 ± 0.22^{ab}	0.76 ± 0.09^{ab}
OK + CF		4.53 ± 0.18^{bcd}	0.75 ± 0.16^{bc}	1.67 ± 0.38^{ab}	0.73 ± 0.14^{ab}
OK^{Pfl} + CF		5.37 ± 0.42^{ab}	0.95 ± 0.21^{ab}	1.79 ± 0.23^{ab}	0.88 ± 0.11^{a}
OK + OK	+FOV	3.84 ± 0.25^{d}	0.27 ± 0.09^{d}	1.29 ± 0.31^{b}	0.51 ± 0.10^{bc}
OK^{Pfl} + OK		4.48 ± 0.29^{cd}	0.52 ± 0.22^{cd}	1.41 ± 0.35^{ab}	0.67 ± 0.08^{bc}
OK + CF		4.79 ± 0.43^{abc}	0.89 ± 0.12^{ab}	1.69 ± 0.26^{ab}	0.62 ± 0.13^{bc}
OK^{Pfl} + CF		5.52 ± 0.59^{a}	1.14 ± 0.10^{a}	1.98 ± 0.29^{a}	0.83 ± 0.15^{a}

Table 2. Variations in NPK and Mg contents in okra leaves both in the absence (-FOV) and presence (+ FOV) of fusarium wilt stress, cultivated with okra (OK) or with cauliflower (CF) as intercropping partner, either with or without *Pseudomonas fluorescens* (Pfl). Data are mean value ± standard deviation, different letters in superscript indicate significant difference as per Tukey's HSD test at ($P \leq 0.05$).

Treatment		Peroxidase (Units/min/g FW) ± SD	Glucanase (Units/min/g FW) ± SD	PPO (Units/min/g FW) ± SD
OK + OK	-FOV	2.09 ± 0.21^{d}	2.37 ± 0.34^{d}	1.35 ± 0.16^{d}
OK^{Pfl} + OK		2.44 ± 0.19^{cd}	2.72 ± 0.42^{de}	2.26 ± 0.27^{be}
OK + CF		2.32 ± 0.35^{d}	2.29 ± 0.59^{d}	1.92 ± 0.13^{bc}
OK^{Pfl} + CF		2.54 ± 0.24^{cd}	3.11 ± 0.25^{cd}	2.05 ± 0.24^{bc}
OK + OK	+FOV	2.88 ± 0.20^{bc}	3.59 ± 0.38^{bc}	1.69 ± 0.21^{cd}
OK^{Pfl} + OK		3.12 ± 0.12^{ab}	4.36 ± 0.27^{ab}	2.43 ± 0.28^{b}
OK + CF		2.92 ± 0.27^{bc}	4.65 ± 0.52^{a}	2.27 ± 0.34^{b}
OK^{Pfl} + CF		3.54 ± 0.41^{a}	5.01 ± 0.36^{a}	3.01 ± 0.4^{a}

Table 3. Variations in the level peroxidase, glucanase and polyphenol oxidase (PPO) in okra leaves both in the absence (-FOV) or presence (+ FOV) of fusarium wilt stress, cultivated with okra (OK) or with cauliflower (CF) as intercropping partner, either with or without *Pseudomonas fluorescens* (Pfl). Data are mean value ± standard deviation, different letters in superscript indicate significant difference as per Tukey's HSD test at ($P \leq 0.05$).

All factors contributed significantly to the shoot length parameter, however a strong ($p < 0.01$) interactive effect FOV × CF was recorded as well. Fusarium wilt stress (+ FOV) caused significant reduction in okra shoot length across all treatments (Fig. 2b). Maximum reduction in shoot length (37%) was recorded when okra was cultivated with okra (OK + OK) without *P. fluorescens* treatment. However, minimum shoot length reduction (10%) was observed in *P. fluorescens* primed okra intercropped with cauliflower (OK^{Pfl}+CF). In the absence of fusarium infection (-FOV), combined effect of *P. fluorescens* priming and cauliflower intercropping resulted in maximum okra shoot length of 18.50 cm, while minimum shoot length (6.74 cm) was recorded in OK + OK treatment in the absence of *P. fluorescens*.

Effect of cauliflower intercropping and Fusarium oxysporum f. sp. vasinfectum on NPK and Mg content in okra leaves

Intercropping with cauliflower and priming with *P. fluorescens* led to an overall enhanced accumulation of primary nutrients (NPK) in okra leaves (Table 1). In the absence of fusarium induced stress (-FOV), significant increase of 27.86 and 86.27% was observed for nitrogen (N) and phosphorus (P) content, respectively, in cauliflower-intercropped okra primed with *P. fluorescens* (OK^{Pfl}+CF), in comparison with primed okra grown with okra (OK^{Pfl}+OK) combination. Among fusarium infected (+ FOV) groups, maximum values of N, P, and K (5.52, 1.14, and 1.98%, respectively) were detected in OK^{Pfl}+CF combination. Minimum content of N and K (3.81 and 1.28%, respectively) was estimated in OK + OK combination without Fusarium inoculation, while P and Mg content was lowest (0.27 and 0.51%, respectively) in OK + OK, grown under wilt stress (Table 2). Overall, NPK and Mg levels remained significantly higher among cauliflower intercropped okra even under disease stress both with and without priming with *P. fluorescens*.

Biochemical analysis

Effect of cauliflower intercropping and Fusarium oxysporum f. sp. vasinfectum on the activity of peroxidase, glucanase and polyphenol oxidase in okra leaves

All individual factors namely CF, FOV and Pfl contributed significantly in the induction of defense related enzymes. However, glucanase activity was significantly upregulated by the interactive effect of FOV and CF. Only in the case of peroxidase a 3-way interactive effect (FOV × Pfl × CF) was observed (Table 1). When infected with fusarium (+ FOV), primed okra cultivated with okra (OK+OK^{Pfl}) showed 8.3% increase in leaf peroxidase contents as compared to OK + OK combination (Table 3). However, highest peroxidase contents (3.54 Units/

min/g FW) were observed when primed okra was cultivated with cauliflower (OK^{Pfl}+CF). Peroxidase content was lowest (2.09 Units/min/g FW) in OK + OK combination. The glucanase contents remained significantly unaltered among all un-inoculated (-FOV) treatments. In contrast, notable glucanse activity (5.01 Units/min/g FW) was detected when bio-protective effect of *P. florescence* was further enhanced with cauliflower intercropping (OK^{Pfl}+CF). However, synergistic effect of both treatments in OK^{Pfl}+CF combination led to significantly highest PPO activity (3.01 Units/min/g FW). Minimum PPO content (1.35 Units/min/g FW) was detected in un-inoculated control (OK + OK) group. Overall, okra priming and intercropping with cauliflower resulted into increased activity of peroxidase, glucanase and polyphenol oxidase in plants under fusarium induced wilt stress.

Effect of cauliflower intercropping and Fusarium oxysporum f. sp. vasinfectum on total chlorophyll content in okra
The interaction between Pfl and CF as well as Pfl and FOV had shown significant ($p < 0.05$) influence on chlorophyll content of okra leaves (Table 1). The bio priming effect of PGPR positively influence level of chlorophyll even under disease stress. Fusarium infection caused significant decrease in okra leaf chlorophyll content across all the treatments (Table 4). In the absence of fusarium wilt stress (-FOV), okra cultivated with cauliflower (OK + CF) as intercropping partner showed higher total leaf chlorophyll content (5.06 mg/g FW) compared to okra cultivated with okra (OK + OK) (4.79 mg/g FW). Under wilt stress, maximum chlorophyll content (3.93 mg/g FW) was recorded in *P. fluorescens* primed okra intercropped with cauliflower (OK^{Pfl}+ CF) compared to rest of + FOV treatments. Minimum chlorophyll content (2.82 mg/g FW) was observed in fusarium infected OK + OK combination.

Disease assessment
A highly susceptible (HS) disease response with 90% disease incidence was observed when okra plants were cultivated with okra (OK + OK) (Fig. 3; Table 5). The application of *P. fluorescens* in OK + OK treatment and the cultivation of okra with cauliflower (OK + CF) as intercropping partner resulted in a substantial decrease in disease incidence (55% and 44%, respectively) as compared to OK + OK combination without *P. fluorescens*. However, okra plants became resistant (R) to fusarium wilt with 20% disease incidence when *P. fluorescens* primed okra was co-cultivated with cauliflower (OK^{Pfl}+CF). There was no significant difference in disease severity when *P. fluorescens* treated okra was cultivated with okra (OK^{Pfl}+OK) as well as okra intercropped with cauliflower (OK + CF) without *P. fluorescens* priming. However, 86% decrease in disease intensity was observed in OK^{Pfl}+CF combination compared to control group (OK + OK).

In vitro analysis

Microconidia germination of Fusarium oxysporum f. sp. vasinfectum in root exudates of okra grown in combination with cauliflower and Pseudomonas fluorescens
Maximum microconidia germination (79.85%) was observed in Potato dextrose broth while minimum in acetate buffer (6.83%), serving as positive and negative control respectively (Table 6). There was significant reduction (23.58%) in germination of microconidia in root exudates of okra intercropped with cauliflower (OK + CF) as compared to okra grown in association with okra (OK + OK). Likewise, a sharp decrease (18.85%) in microconidia germination was recorded when root exudated were extracted from *P. fluorescens* primed okra intercropped with cauliflower (OK^{Pfl}+CF) in comparison with *P. fluorescens* treated okra grown in association with okra (OK^{Pfl}+OK).

In vitro effect of cauliflower and Pseudomonas fluorescens on okra seed germination and radicle length
Okra seeds grown in companionship with cauliflower (OK + CF) in water agar resulted in enhanced seed germination (76.67%) as compared to okra grown with okra (OK + OK) (73.30%). *P. fluorescens* primed okra seeds intercropped with cauliflower (OK^{Pfl}+CF) showed highest seed germination (93.33%). There was no significant difference in okra radicle length in OK^{Pfl}+OK as well as OK^{Pfl}+CF combination. However, minimum radicle length (4.54 cm) was recorded in OK + OK treatment (Table 7).

Treatment		Total Chlorophyll (mg/g FW) ± SD
OK + OK	-FOV	4.79 ± 0.35^{b}
OK^{Pfl}+ OK		4.91 ± 0.16^{b}
OK + CF		5.06 ± 0.51^{a}
OK^{Pfl}+ CF		5.23 ± 0.32^{b}
OK + OK	+FOV	2.82 ± 0.43^{e}
OK^{Pfl}+ OK		3.14 ± 0.17^{de}
OK + CF		3.78 ± 0.27^{cd}
OK^{Pfl}+ CF		3.93 ± 0.19^{c}

Table 4. Effect of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) on total chlorophyll content of okra leaves grown in combination with okra, cauliflower both with and without *Pseudomonas fluorescens* (Pfl). Data are mean value ± standard deviation, different letters in superscript indicate significant difference as per Tukey's HSD test at ($P \leq 0.05$).



Fig. 3. Phenotypic appearance of inoculated *Fusarium oxysporum* f. sp. *vasinfectum* okra plants, shoot and roots. Panel a, b and c represent okra cultivated with okra, dark discoloration of vascular system and infected okra roots, respectively. While panel d, e and f corresponds to okra cultivated with cauliflower, disease free stem and properly growing root system.

Treatment	Disease Incidence (%)*	Disease Severity (%) ± SD**	Disease response
OK + OK	90	70.62 ± 2.27^{a}	HS
OK^{Pfl} + OK	40	19.86 ± 2.08^{b}	MR
OK + CF	50	23.41 ± 1.80^{b}	MR
OK^{Pfl} + CF	20	09.64 ± 2.53^{c}	R

Table 5. Estimation of disease incidence, disease severity and disease response of okra plants inoculated with *Fusarium oxysporum* f. sp. *vasinfectum* grown in combination with okra (OK) and cauliflower (CF), either in the presence or absence of *Pseudomonas fluorescens* (Pfl). *Data evaluated for total number of plants in each treatment. ** Data were mean value ± standard deviation, different letters in superscript indicate significant difference as per Tukey's HSD test at ($P \leq 0.05$).

Discussion

Fungal infection lead to persistent yield losses, thus limit the sustainability of continuous cropping system[50]. There is an urgent need to develop innovative and environment friendly strategies to prevent the dependence on chemical fungicides[51]. Integrated disease management strategies focused on biological approaches offer a promising avenue for fungal induced disease suppression[52]. Pathogenic members of genus Fusarium pose a serious threat to agriculture production. In this context, the present study evaluated the combined effect of

Treatments	Mean number of conidia germinated ± SD	Conidia germination Percentage (%)
Acetate buffer	13.66 ± 1.53	06.83^{f}
Potato dextrose broth	159.70 ± 1.15	79.85^{a}
OK + OK	109.60 ± 2.08	54.80^{b}
OK^{Pfl}+OK	93.67 ± 2.52	46.83^{c}
OK + CF	83.77 ± 0.57	41.88^{d}
OK^{Pfl}+CF	76.01 ± 2.43	38.00^{e}

Table 6. Effect of okra root exudates on microconidia germination of *Fusarium oxysporum* f. sp. *vasinfectum*. Data are mean value ± standard deviation, different letters in superscript indicate significant difference as per Tukey's HSD test at ($P \leq 0.05$).

Treatment	Seed Germination (%) ± SD	Radicle length (cm) ± SD
OK + OK	73.30 ± 1.73 ^{c}	4.54 ± 0.94 ^{b}
OK^{Pfl}+OK	80.01 ± 1.52 ^{b}	10.40 ± 0.57 ^{a}
OK + CF	76.67 ± 2.08 ^{b}	8.90 ± 1.66 ^{a}
OK^{Pfl}+CF	93.33 ± 1.15 ^{a}	11.84 ± 1.95 ^{a}

Table 7. In vitro okra seed germination assay in water agar. Data are mean value ± standard deviation, different letters in superscript indicate significant difference as per Tukey's HSD test at ($P \leq 0.05$).

cauliflower intercropping and bio-priming with *Pseudomonas fluorescens* to mitigate the wilt development in okra.

Intercropping cauliflower with okra positively influenced production of plant biomass. Similar to our findings, enhanced plant growth was also observed in tomato/leek, faba bean/wheat and peanut/*Atractylodes lancea* intercropping combinations even under disease stress[53–55]. Previous studies suggested that spatial arrangement of diverse root systems stabilize crop productivity even in the presence of fungal pathogens[56–61]. Okra biomass enhancement is probably due to exudation of glucosinolate derived metabolites by cauliflower roots. These exudates create a bio-fumigation effect in rhizosphere by suppressing soil inhabiting pathogens and simultaneously optimizing the soil environment for plant growth. There was a sustained growth pattern in *P. fluorescens* primed okra inoculated with pathogen, thus signify the role of the biocontrol agent in mitigating wilt stress.

PGPR are known to promote plant growth by employing diverse mechanisms including phosphate solubilization, siderophore-mediated iron sequestration and secretion of indole-3-acetic acid[62–65]. By decreasing stress induced ethylene levels, *P. fluorescens* prevent the root-stunting response associated with *Fusarium* infection as reported by Khan et al.[66]. Thus, *P. fluorescens* maintained functionality of okra root system for sustained nutrient and water acquisition. Interestingly, highest okra growth was recorded when cauliflower intercropping was integrated with *P. fluorescens* priming. This synergistic interaction represented a bimodal defence strategy where cauliflower metabolites provided chemical suppression of fungal spores[67], while *P. fluorescens* plausibly ensured competitive rhizosphere colonization and activated induced systemic resistance (ISR) in okra[68].

Cultivation of okra with cauliflower introduced diversified root system and resulted in more efficient utilization of soil nutrients[69]. Similarly, our study resulted into enhanced accumulation of NPK and Mg in okra companied with cauliflower. Enhanced nutrition may be attributed to niche complementarity[70]. Zhang et al.[71] reported enhanced nutrient absorption in wheat/maize intercropping system compared to each monocrop. Likewise, Zhu et al.[72] documented improved nutrient uptake in maize/pea cropping system. The minimum phosphorus and magnesium contents were recorded in okra cultivated with okra that might be associated with *Fusarium* induced vascular clogging as described by Gordon[73] or root necrosis that impaired active absorption of these nutrients as documented in previous research[55,74]. Fujikawa et al.[75] reported a positive correlation between magnesium deficiency and host susceptibility to *Fusarium* wilt development.

PGPR secrete organic acids and siderophores to enhance nutrient uptake[76]. However, such nutrient absorption is limited when rhizosphere of host plant is under heavy pathogen load. Contrary to earlier studies[77,78], we noted that rapid colonization of FOV outcompeted *P. fluorescens* and thus rendered bio-fertilization less effective. This phenomenon suggested that beneficial microbes require favourable ecological environment to exhibit bio-fertilization traits under stress conditions. Suppression of FOV plausibly due to root exudates of cauliflower resulted in favourable environment for *P. fluorescens* to grow and facilitate nutrient absorption in host plant. Similar outcomes were reported by Köhl et al.[79] who demonstrated that success of biocontrol mechanism is highly dependent on physiology of host plant. Significantly high magnesium content in *P. fluorescens* primed okra cultivated with cauliflower led to enhanced active transport of nitrogen and potassium and resulted in higher nutrient assimilation. Ecological engineering by adopting intercropping is a pre-requisite to enhance biofertilization potential of *P. fluorescens*[80]. Optimization of nutrient profile not only leads to enhanced growth of host plant but also support fusarium induced stress tolerance[81]. Improved potassium concentration associated with regulation of osmotic balance to suppress the spread of fungal-induced wilt[82]. Likewise, increased

magnesium levels lead towards sustained photosynthetic machinery to prevent chlorosis despite blockage of vascular tissues[83]. Thus, improved nutrition serves as defense framework against fungal spread[84]. These findings are in line with modern agronomic practices where utilization of rhizospheric synergies act as a tool for enhancing crop productivity and resilience against pathogens[85].

Pseudomonas species has been known to effectively suppress fusarium wilt by outcompeting pathogens for nutrients and space through the production of siderophores and antibiotics[86–88]. Additionally, significant upregulation of peroxidase, β- 1,3-glucanse and polyphenol oxidase (POD) suggested the activation of induced resistance in *P. fluorescens* primed okra cultivated with cauliflower. These enzyme act in co-ordination against vascular colonization by fungal infection[89]. β- 1,3-glucanse directly hydrolyze glucan polymers in fungal cell wall and cause cell lysis[90]. Peroxidase and POD concurrently catalyze the oxidation of phenolics into highly toxic quinones. These quinones create unfavorable environment for survival of fungal hyphae[91–95]. POD play its role in scavenging reactive oxygen species and in cross-linkage of lignin polymers. This lignification mechanically reinforces vascular xylem vessels of okra roots[96]. Lignification hinders the entry of pathogens at infection site and blocks the spread of fungal infection[97].

Reduced fungal inoculum along with optimized potential of *P. fluorescens* to elicit induced system resistance, allowed okra to reserve enough energy resources for synthesis of defense enzymes rather than wasting metabolic resources in response to cope pathogen load. The study conducted by Hwang et al.[98] suggested that glucanase genes were only expressed when rice plants get infected with *Magnaporthe grisea*. Our results showed that the glucanase levels remained non-significant in all treatments in the absence of FOV induced infection as a part of energy saving strategy adopted by okra. PGPR alone may not be sufficient enough to rapid hydrolytic response. The presence of diversified rhizosphere due to exudation of cauliflower metabolites and *P. fluorescens* signals that enable okra to deploy maximum glucanase levels in response to stress. Similar results were demonstrated in the study conducted by Senthilraja et al.[99] who recorded higher glucanase activity in treatment containing interaction of groundnut and two biocontrol agents (*P. fluorescens* and *Beauveria bassiana*). In agreement with these findings, higher antioxidant enzyme contents were documented in citrus intercropped with alfalfa[100].

The defense related enzymes like POD, PPO, and glucanase levels were effectively triggered by pathogen and their activity was significantly enhanced by the presence of cauliflower and PGPR highlights their role in augmenting plant self defense mechanism. As we found minimum levels of POD, PPO, and glucanase in okra companied with okra without fungal infection depicts basal metabolic state of plant. In the absence of metabolic signals from pathogen or biocontrol agent, plant maintain low enzymatic activity to conserve resources for primary growth[101]. Previous studies indicated up-regulation in the levels of antioxidant enzymes in the plants cultivated in intercropping system[102,103] as well as bio-primed with PGPR[104,105] under fungal infection. We also found significant increase in the activity of defense enzymes (peroxidase (POD), polyphenol oxidase (PPO) and β−1,3-glucanase) in *P. fluorescens* primed okra companied with cauliflower under FOV induced stress. This upregulation in the activity of the defense related enzymes can be attributed to stimulation of antioxidant as well as pathogenesis related (PR) protein pathways[106]. Kaur et al.[107] also reported upregulation of antioxidant enzymes and PR-proteins in resistant barley infected with *Bipolaris sorokiniana*. Peroxidase and PPO act as essential component of first line of defense by inducing cell wall lignification as hypersensitive response[108] and oxidation of phenols into fungi toxic metabolites (quinones) that inhibit fungal growth at both pre and post-penetration level[109–111].

In this study, higher total chlorophyll content in okra intercropped with cauliflower was in the absence of fusarium induced wilt. According to the findings of Tang et al.[112], higher chlorophyll content in intercropping system is probably due to alteration in rhizosphere signalling and niche differentiation. Similar to our observations, Li et al.[113] and Zhang et al.[114] also demonstrated improved chlorophyll content in maize/peanut and maize/soybean intercropping system, respectively. Root exudates of Brassica crops (cauliflower) act as a soil conditioner by supressing soil pathogens[115] which could divert energy away from biosynthesis of pigments. Hence, intercropped okra invest all the resources in synthesis of light harvesting complexes. Our results indicated a sharp decline in total chlorophyll when okra was companioned with okra under fusarium induced stress. This decline might be due to disruption in vascular transport and ionic balance under fusarium infection. *Fusarium oxysporum* f. sp. *cubense* hyphae block xylem and cause hinderance in uptake of water along with nutrients such as magnesium[116]. As magnesium is central atom in chlorophyll molecule, its deficiency leads to activation of chlorophyllase enzyme that degrade chlorophyll[117]. In our study, chlorophyll content was maximum when effect of companion cropping with cauliflower was further enhanced by priming with *P. fluorescens*. In this treatment, cauliflower likely act as biofumigant that inactivate the pathogen. Simultaneously, the *P. fluorescens* function as a priming agent by modulating hormonal balance of plant. Asif et al.[118] demonstrated that *P. fluorescens* produce cytokinins that delay leaf senescence and stimulate synthesis of chlorophyll. Moreover, *P. fluorescens* induced antioxidant enzymes that actively scavenge reactive oxygen species[119]. Lower ROS levels prevent oxidative degradation of the thylakoid membranes where chlorophyll is present[120,121]. In this way, interaction of intercropping with *P. fluorescens* maintained optimum chlorophyll level even when challenged with fusarium wilt stress.

Chang et al.[122] reported maize/soybean intercropping restricted development of *Fusarium oxysporum* B3S1 with the presence of PGPR. Likewise, Ding et al.[123] stated restricted growth of *Bipolaris maydis* in maize/pepper companion cropping due to the defense metabolites exuded by maize in rhizosphere. Similarly, exudates from okra cultivated with cauliflower suppressed FOV microconidia germination that seems to be involved in reduced disease incidence and severity in okra-cauliflower combination. The wilt incidence was minimum when *P. fluorescens* used as a priming agent in okra-cauliflower system. Decreased disease incidence was attributed to reduced FOV activity in rhizosphere accompanied with the growth promoting potential of *P. fluorescens*.

The reduced germination of FOV microconidia in root exudates of okra intercropped with cauliflower might be due to the shift in composition of okra exudates due to interspecific signals from cauliflower. Zhou et al.[124]

described the shift in root exudates chemistry of tomato leading to stimulated growth of beneficial microbes against *Verticillium dahliae* when companioned with potato-onion (*Allium cepa* var. *agrogatum*). Modified okra exudates might contain organic compounds and phenolics that provide antifungal activity to reduce microconidia germination. The suppression of fungal microconidia germination and mycelial growth may be triggered by the presence of broad spectrum antifungal metabolites in the altered exudates profile of host plant. Yang et al.[125] demonstrated that the modified faba bean exudates contain amino acids that exhibit antifungal potential against *Fusarium oxysporum* f. sp. *fabae* in faba bean-wheat intercropping system. Microconidia germination further dropped when intercropped okra was primed with *P. fluorescens*, might be due to role of modified exudates as precursor for *P. fluorescens* to yield antifungal peptides, in addition to its potential to stimulate ISR[126]. However, additional studies are required to understand the composition of okra root exudates influenced by the PGPR and intercropping agent to link the compounds responsible for disease suppression and plant growth stimulation. Moreover, controlled greenhouse conditions do not simulate filed conditions like temperature fluctuations, soil moisture and competing native soil microbes. Likewise, identification and thresholds of responsible bioactive compounds need to be determined. Although intercropping increase the efficiency of land use and resource utilization but field validation trials at different locations along with high resolution metabolomic profiling of root exudates are mandatory.

Conclusion

Our results demonstrated that while fusarium infection typically leads to stunted growth of okra, the companionship with cauliflower and pre-treatment with *P. fluorescens* significantly alleviated the wilt induced stress. The significant maintenance of okra growth in the intercropping setup in synergy with biopriming agent indicated the activation of a multi-layered plant defense response[127]. However, the enhanced root and shoot vigor observed in the primed treatments may be due to improved hydraulic conductivity and nutrient uptake efficiency[128]. This synergistic approach that involved reducing pathogen infecting potential through intercropping while simultaneously strengthening the host defense via bio-priming resulted in enhanced plant growth even under disease pressure. However, future studies should be performed to understand the alterations induced by intercropping in the root exudate chemistry to better understand its role in plant defense.

Materials and methods

Planting material

Okra seeds of variety Sharmeeli and F1 hybrid of cauliflower were procured from local seed market in Lahore, Pakistan. Okra seeds were surface sterilized by soaking in 70% ethanol for 1 min and in 1% sodium hyochlorite (NaOCl) for 13 min. Surface sterilization of cauliflower seeds was achieved by dipping in 0.5% NaOCl for one minute and thoroughly rinsed. Then the okra and cauliflower seeds were given 8 and 3 washes of distilled water, respectively. The seeds were allowed to dry on blotter paper before sowing[129,130].

Culture acquisition, multiplication and preparation of spore suspension

Pure cultures of *Pseudomonas fluorescens* and *Fusarium oxysproum* f. sp. *vasinfectum* (FOV) were kindly provided by the First Fungal Culture Bank of Pakistan, Faculty of Agriculture Sciences, University of the Punjab, Lahore, under accession number FCBP-PB-0083 and FCBP-EPF-1304, respectively. FOV culture was multiplied by inoculating mycelium from pure culture on Petri plates containing Potato Dextrose Agar supplemented with streptomycin (10 mg/L) to avoid bacterial contamination. Inoculated plates were wrapped with parafilm and incubated at 25 ± 3 °C for 7 days in an incubator[131]. The pathogen identity was confirmed and subsequent sequence was submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and received the accession number as PZ585397. For *P. fluorescens*, freshly prepared Luria Bertani gar plates were streaked with pure culture of *P. fluorescens*. Streaked plates were incubated at 30 ± 2 °C for 24 hours[132]. Spore suspension of FOV was prepared by pouring 3 mL of distilled water on 14 days old culture platesand then scrapping with spatula to ensure harvesting of fungal spores. Obtained suspension was poured in flask by filtering through two layers of muslin cloth. The filtrate was passed through Whatman filter paper no. 1 to filter the remaining mycelia. The suspension was diluted to obtain a spore count of 2×10^7 conidia/mL by using hemocytometer[133].

Suspension of *P. fluorescens* was prepared by inoculating the 100 mL of Luria Bertani broth with loopful of bacterial culture in a 250 mL Erlenmeyer flask. Inoculated broth was placed on a rotary shaker at 100 rpm and 30 ± 2 °C for 48 hours[134]. Cells of *P. fluorescens* were harvested by centrifugation at 10,000 rpm, at 4 °C for 10 min. Bacterial pellet was suspended in sterile distilled water and aabsorbance was measured using spectrophotometer at wavelength of 660 nm where absorbance of 0.9 corresponded to cell density of 1×10^8 Colony Forming Units (CFU)/mL[135].

Seed bio-priming and nursery cultivation

Surface sterilized okra seeds were primed with *P. fluorescens* by dipping the seeds in bacterial suspension (cell density 1×10^8 CFU/mL) on a shaker at 250 rpm at 28 °C for 6 hours[136]. Primed seeds were allowed to dry on blotting paper before sowing in trays[137]. Surface sterilized seeds of okra (primed & unprimed) as well as cauliflower were sown in 128 pockets seedling trays containing peat moss for a period of 30 days. Seedling trays were irrigated as per water requirement[138].

Experimental site and treatment plan

Pot experiment was conducted in a glass house at the research station of Faculty of Agriculture Sciences, University of the Punjab, Lahore (31.4835° N, 74.3357° E). For intercropping of cauliflower and okra, pots were partitioned by vertically placing muslin cloth along the diameter of pots and filling the sterilized sandy loam

soil in the resulting compartments to ensure separation of roots while ensuring permeability of root exudate[139]. The experimental setup consisted of following treatments: (i) Okra (OK) cultivated with okra, (ii) Okra primed with *P. fluorescens* (OK^{Pfl}) and cultivated with OK, (iii) OK intercropped with cauliflower (CF), and (iv) OK^{Pfl} intercropped with CF. All the treatments were either inoculated with *Fusarium oxysporum* f. sp. *vasinfectum* spore suspension (+FOV) or remained uninoculated (-FOV). Each treatment consisted of 5 replicates. All the pots were arranged in completely randomized design. The spatial positioning of pots was systematically rotated in randomized design on weekly basis throughout the duration of experiment to eliminate microclimatic variations.

Pathogen inoculation and seedling transplantation

At the age of 30 days, okra and cauliflower seedlings were removed from seedlings trays. Okra seedling roots were washed with distilled water to remove surface adhering peat moss particles. These roots were cut by using sterilized scissors at 1 cm from end of each root and dipped in fungal suspension for 2 min. These inoculated okra seedlings were then transplanted at 1 inch depth along with uninoculated okra or cauliflower seedlings in different combinations in the partitioned pots as per treatment plan[140]. Growth of both crops was observed for two months in controlled glass house environment at $32 \pm 2^{\circ}C$ and $70 \pm 5\%$ relative humidity. All the pots were irrigated with tap water as per requirement.

Analysis of okra agronomic parameters and leaf nutrient content

Okra plants were harvested 8 weeks post transplantation. Shoot and root length of okra were measured from base to the top node. Both roots and shoots were subjected to 60 °C in an oven till constant weight to measure dry weights[141]. After 7 weeks of transplantation, fully expanded okra leaves were collected from each treatment for determination of nitrogen, phosphorus and potassium (NPK) contents. Collected leaf samples were oven dried at $75 \pm 5°$ C for 24 h and ground in Thomas Wiley mill[142]. Leaf nitrogen content was determined by micro kjeldahl method through sample digestion in acid followed by distillation and titration[143]. For determination of potassium, phosphorus and magnesium, ground leaf powder was subjected to 500 °C for 6 h and extracted using acid mixture ($HNO_3 + HClO_4 + H_2SO_4$). Phosphorus content was measured using colorimetric vanadomolybdate method at 420 nm[144]. Potassium content was measured using flame photometer[145]. Leaf magnesium content was determined by EDTA titration technique[146].

Determination of peroxidase, glucanase and Polyphenol oxidase content

To prepare enzyme extract for quantification of peroxidase and glucanase, 0.5 g of leaf samples from 21 days old okra plants were crushed with 3mL of phosphate buffer in cold pestle and mortar. A mixture of 10 mL phosphate buffer and 3 mL of extract was prepared in falcon tubes and centrifuged at 6000 rpm for 10 min at 4 °C. Supernatant was stored at −20° C[138]. For determination of polyphenol oxidase, 10 g leaf samples were boiled for 10 min in 95% boiling ethanol followed by Soxhlet extraction with 75% ethanol. Residue was dissolved in 10 mL of 50% iso-propanol, acting as leaf extract[147]. For determination of peroxidase content, 0.1 mL of enzyme extract was mixed with 20 mM guaiacol, 40 mM H_2O_2, and 50 mM potassium phosphate buffer (at pH 5). Optical density of the mixture was measured in spectrophotometer at 470 nm at the interval of 20 s. A unit of peroxidase activity was defined by measuring changes in value of optical density per minute[148]. For determination of β 1,3-glucanase activity, laminarin was used as the substrate. The reaction mixture contained 250 µL of crude sample and 1% (w/v) substrate in 250 µL of 50 mM acetate buffer (pH 5.5) and incubated for 30 min at 50 °C in a water bath. Readings of spectrophotometer were recorded at 550 nm. One unit of β 1,3-glucanase activity was defined as release of 1 µmol of glucose from laminarin in one minute[149]. Determination of poly phenol oxidase (PPO) activity was carried out by preparation a reaction mixture consisting of 0.005 mL of enzyme extract and 2.95 mL of 20mM catechol solution (prepared in 0.1 M phosphate buffer with pH 6.0). PPO activity as measured in terms of quinone formation rate by measuring absorbance at 420 nm. One unit of enzyme activity was defined as the amount of enzyme that caused an absorbance change of 0.001 min^{-1}[150].

Determination of total chlorophyll content

Total chlorophyll content in okra leaves was determined by dipping 1 g of leaf tissue in 10 mL of 80% acetone and incubating the mixture for 24 h under dark. Total chlorophyll released in acetone was measured by recording absorbance of at 645 and 663 nm for chlorophyll a and b, respectively. Findings were expressed as mg chlorophyll/g of leaf fresh weight[151].

Spectrophotometer absorbance readings were put in following equations to determine chlorophyll a, b, and total chlorophyll content.

$$\text{Chlorophyll a } (\text{mg/g FW}) = \frac{12.7\ (\text{A663nm}) - 2.69\,(\text{A645nm})}{1000}$$

$$\text{Chlorophyll b } (\text{mg/g FW}) = \frac{22.9\ (\text{A663nm}) - 4.68\ (\text{A645nm})}{1000}$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Where:

A663 nm = Absorbance at wavelength of 663 nm.

A645 nm = Absorbance at wavelength of 645 nm.

Disease incidence and severity in fusarium infected okra plants

Disease incidence was determined by measuring number of fusarium infected okra plants and divided by total number of okra plants per treatment to calculate the percentage of infected plants. Following formula was used to calculate disease incidence[152]. Both disease incidence and severity were measured once before harvesting plants, to determine the cumulative effect of treatments on disease suppression. For assessment of disease severity, a rating scale with ratings range from 0 to 5 was used where 0 = no symptoms, 1 = few brownish streaks of vascular darkening, 2- two or more streaks visible on vascular tissue, 3 = clearly visible dark brown discoloration of xylem, 4 = discoloration not only confined to xylem but also on adjacent tissue and 5 = death of plant due to severity of infection[153].

$$\text{Disease incidence } (\%) = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{X}100$$

Disease severity as proportion of fusarium infected stem length to total length of okra stem, determined by following formula[154]:

$$\text{Disease severity } (\%) = \frac{\text{Length of infected stem (cm)}}{\text{Total length of stem (cm)}} \times 100$$

Extraction of okra root exudates

Okra plants were gently uprooted from pots and roots were washed with running tap water to remove surface adhering soil particles. Exudates were extracted by dipping the roots in acetate buffer (25mM, pH = 5.5) for 6 h. Concentration of root exudates was adjusted in the acetate buffer to 20 mL g^{-1} of root fresh weight. Exudates were filtered through 2 layers of sterile muslin cloth and preserved at −80 till future use[155].

In vitro effect of okra root exudates on microconidia germination of *Fusarium oxysporum* f. sp. *vasinfectum*

In vitro evaluation of impact of root exudates of okra grown in different combinations with cauliflower and *Pseudomonas fluorescens* was performed in a 96- well microtiter plate. For each exudate, there were 5 replications. 175 µL of root exudates and 35 µL of fusarium suspension (1×10^7 microconidia/mL) was poured to each well in plate. Plate was incubated in dark, on a rotary shaker for 21 h at 200 rpm. Calculation of germination rate was performed by counting 200 microconidia under optical microscope at 40X. Conidia with protruding germ tube were considered germinated. Wells containing potato dextrose broth were considered as positive while acetate buffer as negative control in the germination assay[156].

In vitro seed germination and radicle length assay of okra

In vitro germination test of okra seeds was performed on 2% water agar. The surface sterilized okra and cauliflower seeds either with or without *Pseudomonas fluorescens* priming were placed on water agar plates. 10 seeds of each okra and cauliflower in triplicate were grown in petri plates in different combination such as okra grown with okra, okra grown with cauliflower, okra primed with *P. fluorescnens* and grown with okra as well as with cauliflower. Plates were kept at 25 °C and observed for a period of 7 days. After 10 days radicle length was measured for okra seeds in all the treatment[157].

Statistical analysis

Statistical calculations and data analysis was performed using Statistix (version 10) analytical software (https://www.statistix.com/). All the results were subjected to three-way analysis of variance (ANOVA), using *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), *Pseudomonas fluorescens* (Pfl) and cauliflower (CF) as main factors. Data were checked for homogeneity of variances using Levene's test. Percentage data was log transformed. Tukey's honestly significant difference (HSD) test with significance level at $p \leq 0.05$ was used to compare treatment means.

Data availability

Datasets generated during or analyzed during the current study are available in the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Plant guideline statement
All methods were carried out in accordance with relevant guidelines.

Additional information
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