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ORIGINAL PAPER

Comparison between Some Biotic and Abiotic Inducers in Controlling Peanut Pod Rots and Aflatoxin Contamination

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ABSTRACT

Peanuts are the fourth most widely cultivated oilseed in the world. Peanut pod rots disease and aflatoxin contamination are significant challenges that affect both production and quality, leading to considerable yield losses and complicating control efforts. Applying fungicides is one of the management techniques that are crucial for preventing peanut pod rot, but it also poses a risk to the health of people and animals and contributes to environmental contamination, thus it is imperative to discover alternatives to fungicides. To follow this path, the efficacy of biotic inducers (*Pseudomonas fluorescens* and *Bacillus subtilis*) and abiotic inducers (bion at concentrations 2, 4, and 8 mM and chitosan at concentrations 1, 2, and 3 mM) in lowering the incidence of pod rots and aflatoxin contamination was evaluated in comparison to fungicide (check control). All tested inducers led to significantly reduce the incidence of pod rot disease and aflatoxin contamination compared to untreated controls, whether under artificial or natural inoculation conditions. In general, chitosan at a concentration of 3 mM, followed by chitosan at a concentration of 2 mM and *P. fluorescens* had the most significant effect in reducing pod rot diseases and aflatoxin contamination, under greenhouse conditions or during the 2022 and 2023 consecutive seasons. Increasing the chemical inducers concentration enhanced their effectiveness in reducing disease incidence. The study also showed a positive relationship between induced resistance and some biochemical changes in peanut pod tissues. These changes included increased phenolic compound, activity of oxidative enzymes (PO, PPO and CAT), content of crude protein and total free amino acids. Furthermore, there was an increase in the activity of various classes of pathogenesis-related (PR) proteins. The data obtained clearly showed that some inducer treatments were able to achieve efficiency close to that of fungicides in reducing peanut pod rot and amount of aflatoxin, this encourages the use of these inducers instead of fungicides.

Keywords: *Pseudomonas fluorescens*, *Bacillus subtilis*, Chitosan, Bion, inducer resistant, biochemical changes, oxidative enzyme, phenols, PR-proteins

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) conceder one of the most sources of protein and oil in the world. It is planting at 30.65 million hectares across more than 100 countries, producing over 51 million tons (USDA, 2024). In Egypt, peanuts are significant crops for both export and local consumption. The cultivated area is 61,442 hectares (153,605 feddans) and the production is 218,835 tons (USDA, 2024). Pod rot is one of the major plant diseases impacting peanut production and quality. It causes substantial productivity losses and poses significant challenges to effective control (Mahmoud *et al*. 2014 and Liu *et al*. 2024).

The prevalence and severity of pod rot have led to annual yield reductions, with affected fields experiencing up to a 15% loss, while severely infected areas may suffer yield losses of up to 50%. In extreme cases, the disease can result in total crop failure (He *et al*. 2022). Pod rots of peanut can be caused by a number of different organisms including *F. moniliforme*, *M. phaseolina*, *R. solani*, *S. rolfsii* and *Pythium spp*. (Mahmoud *et al*. 2014 and 2015 and Liu *et al*. 2024). In the same time, pre-harvest aflatoxin contamination is one of the biggest challenges facing peanut producers (Mahmoud *et al*. 2015). *Aspergillus flavus* and *A. parasiticus* were the most common fungi infected peanut before harvest (Mahmoud *et al*. 2015, Bediako *et al*. 2019 and Yousef, *et al*. 2022). Managing pod rots has been challenging, partly due to the broad host range of the pathogens and the lack of resistant cultivars (Mahmoud *et al*. 2015, He *et al*. 2022 and Liu *et al*. 2024).

According to Walters *et al*. (2007) and Mahmoud *et al*. (2021), induced resistance is the process of active resistance that is reliant on the physical and chemical barriers of the host

plant and is triggered by biotic or abiotic causes. Various chemicals can be act a various points of the plant defense and induce resistance in plants *e.g.*, Butyric acid, Chitosan, Ethylene, and Salicylic acid. The success of systemic resistance depends on the rapid stimulation of the plant's defense processes after infection. These responses include phenolic compound, phytoalexins, lignification, the activation of PR-proteins and induce enzyme activation *e.g.*, PO, PPO, CAT, and chitinase (Tuzun and Kloepper 1994, Hussien, 2011, Abdel Aal *et al.* 2012, Mahmoud *et al.* 2021). Several researchers have shown that induction of resistance in peanut plants resulted in changes in the activity of enzymes such as chitinase, peroxidase, catalase, polyphenol oxidase, and the amount of total protein and phenolic compounds. (Meena *et al.* 2001, Hussien, 2011 and Mahmoud *et al.* 2015 & 2021).

The two main genera of plant growth-promoting Rhizobacteria (PGPR) are Bacillus and Pseudomonas (Karunanithi *et al.* 2000, Meena *et al.* 2001, and Mahmoud *et al.* 2021). In a variety of hosts, including Arabidopsis, carnation, bean, radish, cucumber, chili, grapevine, tomato, tobacco, and peanut, non-pathogenic rhizobacteria can cause systemic resistance against bacteria, fungi, and viruses (Van *et al.* 1998, Verhagen *et al.* 2010, Gruau *et al.* 2015, Jayapala *et al.* 2019 and Mahmoud et al. 2021). When induced by particular bacterial strains, which vary in their capacity to generate resistance, plants have demonstrated diversity in the expression of ISR. According to Van *et al.* (1998), Altinok *et al.* (2013), and Mahmoud et al. (2021), lipopolysaccharides, siderophores, SA, and PR-proteins are specific bacterial components of ISR.

The studies were conducted to verify the effectiveness of some environmentally safe methods for reducing peanut pod rots and their amount of aflatoxin, compared to Balear fungicide as a check control.

MATERIALS AND METHODS

The isolates fungi which used in the study were isolated by the authors from diseased peanut pods and their pathogenic potioniallty were determined (Mahmoud *et al.* 2015).

1. Fungal inocula preparation:

Inocula of *Fusarium moniliforme*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Aspergillus flavus* and *Aspergillus parasiticus* were prepared according to Mahmoud *et al.* (2015).

2. Soil infestation:

Two different methods were used for soil infestation with the tested fungal pathogens in this study:

(A): For pod rot study inoculums of *F. moniliforme*, *M. phaseolina*, *R. solani* and *S. rolfsii* were mixed well with the topsoil of each pot, at a rate of 2% w/w, and the soil was then covered with a thin layer of sterile soil. The pots were irrigated and left for 10 days before sowing.

(B): For study aflatoxigenic fungi, 15 days before sowing each kilogram of soil was infested with 10 ml conidial suspension ($4x10^6$ spores/ml) of *A. flavus* or *A. parasiticus*, and their mixture and spraying with the same concentration of spore suspension after 30 days from sowing.

3. Source of Biotic Inducers:

Mahmoud *et al.* (2021) generously supplied *Bacillus subtilis* and *pseudomonas fluorescens*. The Unit of Identification of Microorganisms Plant Pathology Research Institute (ARC) used the Biolog technique (Biolog, Inc., 3938 Trust Way, Hayward, CA94545, and USA) to validate the identification.

4. Preparation of bacterial inoculum

suspensions of bacteria (1×10^6 CFU/mL) were prepared according to the method as described by Mahmoud *et al.*(2021).

4. Treatments applied:

To reduce the prevalence of pod rots and aflatoxigenic fungi, biotic inducers *P. fluorescens* and *B. subtilis* were used in conjunction with abiotic inducers Bion at 2, 4, and 8 mM and chitosan at 1, 2, and 3 mM. Twenty and forty days after planting, the treatments were sprayed on the leaves. Fifty days after seeding, 7.5 liters of Balear fungicide SC 50% (Chlorothalonil with a PHI of 10 days) were treated per feddan.

5. Disease assessment:

(A): At harvest, incidence of pod rot was recorded. According to apparent symptoms four categories of pod rots (dry brown lesion, pink discoloration and General breakdown), in addition

to healthy pods, were adopted according to **Satour *et al.*, (1978)**

Pod rot categories (%) $= \frac{\text{No. of rotted pod}}{\text{No. of total pods}} \times 100$

(B): Aflatoxigenic fungi associated with peanut pods were isolated after harvesting following the method described by Garren and Porter (1970). The isolates were defined according to Marne and Johan (1988).

6. Determination of aflatoxins:

Aflatoxins were extracted and determined according to **A.O.A.C. (2000).** The concentrations were estimated by external calibration using a single level height near the desired height of the sample under test.

The following equation was used for calculation:

$$Cs = \frac{V}{Va} \times \frac{Vf}{W} \times \frac{h_{sam}}{h_{st}} \times Y$$

V= Extraction volume (ml)
V_a = Aliquot taken from extraction volume (ml)
V_f= Final dilution volume (ml)
h_{sam}= The height of sample peak
h_{st}= The height of standard peak
Y = Standard concentration (ng/ml).
W = Weight of sample taken for test (g).

7. Biochemical changes associated with induced resistance:

Peanut samples (primordial pods) were collected after five days from second foliar spraying and abstracted according to Goldschmidt *et al*. (1968). The activities of oxidative enzymes *i.e.,* catalase (CAT), polyphenol oxidase (PPO), and peroxidase (PO), were determined by Maxwell and Bateman (1967), Matta and Dimond (1963), and Allam and Hollis (1972) respectively and assayed using a spectrophotometer at 240, 495 and 425 nm., respectively. The reaction substrate for each oxidizing enzyme was catechol, pyrogallol and H_2O_2 to determine the activity of PPO, PO and CAT, respectively. Additional samples were extracted in Soxhlet units with 75% ethanol for 10–12 h and then used to determine phenols, percent crude (%) and total free amino acids, as described by Snell and Snell (1953), A.O.A.C. (1998) and Moore and Stein (1954) respectively. The contents of phenolic compounds and total free amino acids were calculated as milligram equivalents of catechol and arginine per gram of fresh weight of peanut tissue, respectively.

8. Sodium Dodecyl Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

Changes in soluble proteins due to treatment with inducer materials were determined in peanut primordial pods using the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique described by Broglie et al., (1986). Vertical slab gels (0.75 mm/thick) were cast and electrophoresed using the Bio Rad Mini-Protean II system. The gel was stained with Coomassie Brilliant Blue R-250 solution, photographed, and scored using a gel imaging system produced by Alpha Ease FC (Alphaimager 2200) in the United States. Similarity matrices were generated using Gel works 1D advanced software UVP-England Program.

9. Greenhouse experiment:

Ten seeds were sown in sterilized plastic pots of 50 cm diameter, containing sterilized sandy-clay soil at a ratio of 2:1, which had been pre-sterilized by steam for 2 h. Each treatment included four replicates, and the pots were arranged in the greenhouse according to a randomized block design. Giza 6 peanut cultivar (sterilized seeds) were sown in potted soil containing inoculated with approximately 100 g (2% w/w) of a manually prepared mixture of *M. phaseolina*, *R. solani*, *S. rolfsii*, and *F. moniliforme* to study the effect of inducer material on pod rot incidence or artificial inoculation by *Aspergillus flavus* and *A. parasiticus* separately and their mixtures to study the effect of inducer material on occurrence of aflatoxigenic fungi and the content of aflatoxin contamination. The inducer materials were applied as described before, and diseases' incidence was determined as mentioned before.

10. Field experiment:

Throughout the field trials, which were carried out in a naturally occurring field afflicted with causative diseases at the Ismailia Experimental Station of (ARC) during the 2022 and 2023 growing seasons, peanut seeds, cv. Giza 6, were utilized for sowing. The soil type was sandy loam, with a pH of 7.98, an EC of 7.2, 77% sand, 11% silt, and 12% clay. Seeds were sown with 10 cm between plants during the first week of May. Three repetitions and a randomized block design were used to set up the experiment. The experimental unit has an area of 10.5 m² (1/400 area). The recommended agricultural procedures were followed. Each plot's plants are excavated and turned upside down after they reach their ideal age. Pods were weighed after being threshed and let to air dry for three days. Induced materials were applied as previously described, and disease incidences were assessed as mentioned before.

11. Statistical analysis:

Data were statistically analyzed using analysis of variance (ANOVA) by using statistical analysis program "COStat 6.4" (CoStat, 2005). Mean differences were separated using the least significant difference (LSD) test at a significance level of $P < 0.05$.

RESULTS

1. Greenhouse experiments:

1.1. Effect of different inducer treatments on peanut pod rots incidence:

Data presented in Table (1) show that, the inducer treatments, weather biotic or abiotic, at different concentrations resulted in a significant reduction in the incidence of peanut pod rot (%) compared to the control treatment. In the case of abiotic inducers, chitosan at all concentrations was more effective in reducing disease incidence while, *P. fluorescens* was more efficacious for biotic inducers. Chitosan at concentrations of 3 mM and 2 mM recorded the highest reduction in all types of pod rots incidence, followed by *P. fluorescens*, compared to other inducer treatments.

However, fungicide treatment recorded the highest effect in reducing of pod rot incidence. The data also revealed a relationship between concentrations of abiotic inducers and their effectiveness in controlling of pod rot incidence where, increasing the concentration of abiotic materials (Chitosan and Bion) led to an increased in their effect (Table 1).

Table (1): Effect of different inducer treatments on pod rots incidence under artificial greenhouse conditions.

Treatment	Conc. (mM)	Disease incidence [z)]			Apparent healthy pods
		Dry brown lesion	Pink discoloration	General Breakdown	
Bion	**2 mM**	14.24	2.37	16.44	66.95
	4 mM	13.29	3.26	15.37	68.08
	8 mM	11.53	3.04	13.73	71.70
Chitosan	**1mM**	13.60	1.81	15.80	68.79
	2 mM	11.27	2.03	12.13	74.57
	3 mM	9.74	1.15	9.94	79.17
P. fluorescens		11.67	2.43	11.87	74.03
B. subtilis		12.22	1.74	14.42	71.62
Balear fungicide		6.71	1.15	8.91	83.23
Control		17.70	4.13	19.38	58.79
L.S.D. 5%:		2.272	0.633	2.173	2.411

1.2. Effect of different inducer treatments on the occurrence of aflatoxigenic fungi and aflatoxin content:

The data in Table 2 show that the occurrence of aflatoxin-producing fungi was generally affected by inducer

treatment. The frequency of *A. flavus* was higher than that of *A. parasiticus*, whether inoculated alone or in combination with *A. parasiticus*. An increase in the concentration of abiotic inducers corresponded to decrease the occurrence of aflatoxigenic fungi.

Chitosan at 3 mM concentration recorded the lowest frequency of aflatoxin fungi and aflatoxin content in seeds compared to other treatments, whether aflatoxin fungi were inoculated alone or in combination, and its effect was also better than that of fungicide treatments. Conversely, the highest aflatoxin content was recorded with *B. subtilis* treatment, whether *A. flavus* and *A. parasiticus* were inoculated individually or in combination, compared to other inducer treatments (Table 2).

Table (2): Effect of different inducer treatments on frequency of *A. flavus, A. parasiticus* and aflatoxin content in seeds under artificial greenhouse conditions.

Treatments	Conc. (mM)	*A. flavus*			*A. parasiticus*			*A. flavus* + *A. parasiticus*			
		* A.f (%)	aflatoxin (ppb)		**A.p (%)	aflatoxin (ppb)		A.f (%)	A.p (%)	aflatoxin (ppb)	
			B_1	B_2		B_1	B_2			B_1	B_2
Bion	2 mM	40	120	80	30	100	45	40	30	170	90
	4 mM	20	100	Nd	20	Nd	Nd	30	30	110	Nd
	8 mM	20	70	Nd	10	Nd	Nd	20	10	90	Nd
Chitosan	1mM	30	80	50	20	70	30	30	20	100	70
	2 mM	20	50	Nd	10	Nd	Nd	20	10	70	Nd
	3 mM	10	30	Nd	0	Nd	Nd	10	10	50	Nd
P. fluorescens		30	110	40	20	100	Nd	40	30	150	70
B. subtilis		40	140	80	30	120	60	50	30	210	90
Balear fungicide		20	80	Nd	20	30	Nd	25	20	100	Nd
Control		40	220	100	30	200	100	50	40	240	120

***A.f = Frequency of *A. flavus* **A.p = Frequency of *A. Parasiticus* ***Nd= Non detected**

2. Field experiments:

2.1. Effect of different inducer treatments on peanut pod rots incidence:

Presented results in Table 3 illustrated that, inducer treatments (biotic and abiotic) at the tested concentrations had significant effects in reducing pod rot diseases compared to control in both 2022 and 2023 seasons.

The presented data confirmed that, increasing the concentration of abiotc inducers leads to an increase in their effect in reducing the incidence of peanut pod rot (%). In this regard, the highest reduction in pod rot incidence (%) was achieved during both seasons with chitosan at 3 mM and 2 mM, followed by *P. fluorescens*, although fungicide treatment was generally the most effective. On the other hand, *B. subtilis* had the least effect on peanut pod rot incidence (%) during the 2022 and 2023 growing seasons.

2.2. Effect of different inducer treatments on occurrence of aflatoxigenic fungi and amount of aflatoxin:

Data obtained in (Table 4) showed the occurrence of aflatoxigenic fungi and the amount of aflatoxin in seeds had been affected by treatment with biotic and abiotic inducer materials. Increase the concentrations of abiotic inducers (chitosan and Bion) led to decreasing the occurrence of aflatoxigenic fungi in seeds during both of growing seasons 2022 and 2023. *B. subtilis* treatment recorded the highest occurrence of aflatoxigenic fungi and the greatest amount of aflatoxin in seeds.

On the other hand, the best treatments for reducing the occurrence of aflatoxiginec fungi and amount of afltoxins in seeds were achieved by chitosan at 3 mM, compared to other treatments, including the fungicide treatment, in both of growing seasons 2022 and 2023.

2.3. Effect different inducer treatments on peanut pods yield:

Peanut pod yield during the 2022 and 2023 growing seasons differed considerably between the investigated inducer treatments and fungicide, according to data in Table 5.

In both seasons, *P. fluorescens* and chitosan at 3 mM produced the maximum peanut pod yield, while Bion at 2 mM produced the lowest. However, over the two growing seasons of 2022 and 2023, the best pod yield production was obtained with fungicide treatment.

3. Biochemical changes associated with different inducer treatments:

3.1. Effect of different inducer treatments on phenol contents:

As demonstrated by the results in Table 6, plants treated with the tested inducer treatments had significantly higher levels of phenolic compounds (free, conjugated, and total phenols) than the control .

Table (3): Effect of different inducer treatments on pod rots incidence under field conditions during 2022 and 2023 seasons.

Treatment	Conc. (mM)	Season 2022				Season 2023			
		Dry brown lesion	Pink Discoloration	General breakdown	Apparent Healthy Pods	Dry brown lesion	Pink discoloration	General Breakdown	Apparent healthy pods
Bion	2 mM	11.94	1.87	13.79	72.40	11.99	1.65	12.92	73.44
	4 mM	11.22	0.89	13.17	74.72	10.27	0.66	12.30	76.77
	8 mM	10.49	0.48	11.66	77.37	9.03	0.23	11.07	79.67
Chitosan	1 mM	10.37	1.23	12.32	76.08	8.42	1.01	10.45	80.12
	2 mM	10.14	0.57	10.89	78.40	8.19	0.35	10.02	81.44
	3 mM	8.64	0.00	9.46	81.90	7.69	0.00	8.83	83.48
P. fluorescens		9.98	0.75	12.94	76.33	8.54	0.00	9.79	81.67
B. subtilis		12.08	0.98	13.04	73.90	10.13	0.68	12.17	77.02
Balear fungicide		7.77	0.35	8.20	83.68	6.82	0.00	7.07	86.11
Control		13.00	2.01	15.40	69.59	13.04	1.98	13.23	71.75
L.S.D. 5%:		2.336	0.366	3.013	4.315	1.591	0.231	2.391	3.681

Table (4): Effect of different inducer treatments on the frequency of *A. flavus, A. parasiticus* and aflatoxin content in seeds under field conditions during the 2022 and 2023 growing season.

Seasons		2022				2023			
Treatments	Conc. (mM)	*A. flavus* (%)	*A. parasiticus* (%)	Aflatoxin (ppb)		*A. flavus* (%)	*A. parasiticus* (%)	aflatoxin (ppb)	
				B_1	B_2			B_1	B_2
Bion	**2 mM.**	30	20	200	70	20	10	150	40
	4 mM.	20	20	150	20	10	10	120	30
	8 mM.	10	0	80	Nd	10	0	40	20
Chitosan	**1mM.**	20	10	110	20	10	10	80	40
	2 mM.	10	0	40	Nd	10	0	20	Nd
	3 mM.	10	0	20	Nd	0	0	Nd	Nd
P. fluorescens		20	10	140	Nd	20	15	110	Nd
B. subtilis		30	20	250	70	25	20	200	70
Balear fungicide		10	10	120	Nd	10	10	80	Nd
Control		40	30	360	130	35	20	270	85

***A. *flavus* = Frequency of *A. flavus* **A. *Parasiticus* = Frequency of *A. Parasiticus* *Nd= Non detected

Accordingly, when compared to the other treatments, peanut plants treated with chitosan at 3 mM had the highest phenol level, followed by *P. fluorescens*, while those treated with Bion at 2 mM had the lowest phenol content. The data unequivocally demonstrated that the amount of phenols in peanut primordial pods increased as the concentration of chemical inducers increased.

3.2. Effect of different inducer treatments on oxidative enzymes:

The illustrated data in Table 7 showed that, treated with inducer material increased the activity of oxidative enzymes *e.g.* (PO), (POP), and (CAT), in peanut primordial pods compared to untreated control.

In particular, chitosan at 3 mM Followed by *P. fluorescens* give the highest increase in activity of PO, PPO and CAT. Moreover, increasing the concentration of chemical inducers was associated with a corresponding incensement of the enzyme's activity.

Table (5): Effect of different inducer treatments on total peanut pod yield under field conditions during two successive seasons 2022 and 2023.

Treatments	Conc. (mM)	Season 2022		Season 2023	
		Yield (Ton)	Loss of yield (%)	Yield (Ton)	Loss of yield (%)
Bion	2mM	1.264	12.06	1.220	11.31
	4mM	1.380	22.34	1.320	20.44
	8mM	1.428	26.60	1.376	25.55
Chitosan	1 mM	1.540	36.52	1.520	38.69
	2 mM	1.556	37.94	1.526	40.22
	3 mM	1.640	45.39	1.592	45.26
P. fluorescens		1.572	39.36	1.538	40.33
B. subtilis		1.540	36.52	1.504	37.23
Balear fungicide		1.704	51.06	1.672	52.55
Control		1.128	0.00	1.096	0.00
L.S.D. 5%:		0.075		0.083	

Table (6): Effect of different inducer treatments on phenol contents (mg/g fresh weight) in peanut primordial pods.

Treatments	Conc. (mM)	Phenol content		
		Total	Free	Conjugate
Bion	2 mM	7.73	4.94	2.79
	4 mM	9.13	5.99	3.14
	8 mM	9.28	7.19	2.09
Chitosan	1 mM	8.35	5.81	2.54
	2 mM	9.66	6.52	3.14
	3 mM	12.14	9.81	2.33
P. fluorescens		11.74	6.15	5.59
B. subtilis		9.82	6.67	3.15
Balear fungicide		7.45	4.86	2.59
Control		6.38	3.95	2.43
L.S.D. 5%:		0.712	0.593	0.351

3.3. Effect of different inducer treatments on total free amino acids and protein content %:

Total free amino acids increased significantly as a result of the inducer treatments in comparison to the control treatment, according to the results displayed

in Table 8. During the growing seasons of 2022 and 2023, the concentration of chemical inducers increased in tandem with the total amount of free amino acids. Chitosan at 3 mM had the greatest concentration of total free amino acids among the evaluated treatments. On the other hand, Bion recorded the lowest total free amino acids in both seasons at 2 mM.

In seasons 2022 and 2023, the crude protein content of peanut primordial pods increased with all inducer treatments when compared to the untreated control (Table 8). The rate of crude protein content increased as the concentration of chemical inducers increased. In this regard, the highest crude protein content was produced by *P. fluorescens* with chitosan at 3 mM during the two growing seasons of 2022 and 2023, while the lowest content was recorded by Bion at 2 mM. In this regard, the highest crude protein content was produced by *P. fluorescens* with chitosan at 3 mM during the two growing seasons of 2022 and 2023, while the lowest content was reported by Bion at 2 mM.

Table (7): Effect of different inducer treatments on peroxidase (PO), polyphenoloxidase (PPO) and catalase (CAT) activity in peanut primordial pods.

Treatments	Conc. (mM)	Enzyme activity		
		PO	CAT	PPO
Bion	**2 mM**	0.379	0.076	2.021
	4 mM	0.655	0.124	3.909
	8 mM	1.032	0.134	4.654
Chitosan	**1 mM**	0.693	0.087	3.375
	2 mM	1.136	0.176	4.384
	3 mM	1.974	0.297	6.192
P. fluorescens		1.690	0.188	5.161
B. subtilis		1.369	0.103	4.549
Balear fungicide		0.377	0.090	2.538
Control		0.368	0.061	1.786
L.S.D. 5%:		0.353	0.223	0.551

Table (8): Effect of different inducer treatments on total free amino acids and percentage of protein content in peanut primordial pods.

Treatments	Conc. (mM)	Free amino acids (mg/g fresh weight)	Protein content (%)
Bion	**2 mM**	0.540	7.485
	4 mM	0.544	8.770
	8 mM	0.653	12.620
Chitosan	**1 mM**	0.513	7.715
	2 mM	0.683	10.515
	3 mM	0.836	16.435
P. fluorescens		0.791	15.440
B. subtilis		0.737	14.100
Balear fungicide		0.472	7.255
Control		0.366	6.820
L.S.D. 5%:		0.046	2.031

3.4. Effect of inducers treatments on soluble proteins in peanut primordial pods:

Data presented Table 9 and Figure 1 indicates the emergence of new protein bands as a result of inducer treatments.

Specifically, chitosan treatment in all concentrations showed participation in a new protein band with molecular weights (MW) 55 and 48 KDa. Additionally, chitosan at 2 mM and 3 mM, as well as *P. fluorescens* treatment, induced the formation of a new protein bands with MW 39 KDa. Notably, chitosan at 3 mM uniquely produced a new band with 22 KDa.

On the other hand, a protein band (93 KD) was present in all treatments including the untreated control, except for chitosan-treated plants. Moreover, all treatments and untreated control participated to protein bands with MWs of 22, 17 and 16 KDa.

These results confirmed by the dendrogram analysis (Figure 2), highlights the similarity between protein groups. Chitosan treatments were grouped into a single one subgroup, with chitosan at 3 mM being unique. In contrast, the other inducer treatments were clustered together in a separate subgroup, distinctly distant from the fungicide treatment and the untreated control, which were clustered separately in their own subgroup.

Table (9): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins extracted from peanut primordial pods growing under artificial inoculation conditions.

Molecular weight (KD)	Chitosan			Bion			*Bacillus subtilis*	*Pseudomonas fluorescens*	Fungicide	Control
	Ch1	Ch2	Ch3	Bi1	Bi2	Bi3	B.s.	P.f.	Fun.	Cont
93	0	0	0	1	1	1	1	1	1	1
55	1	1	1	0	0	0	0	0	0	0
48	1	1	1	0	0	0	0	1	0	0
39	0	1	1	0	0	0	0	0	0	0
27	1	1	1	1	1	1	1	1	1	1
25	1	0	0	1	1	1	1	0	1	1
22	0	0	1	0	0	0	0	0	0	0
17	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1

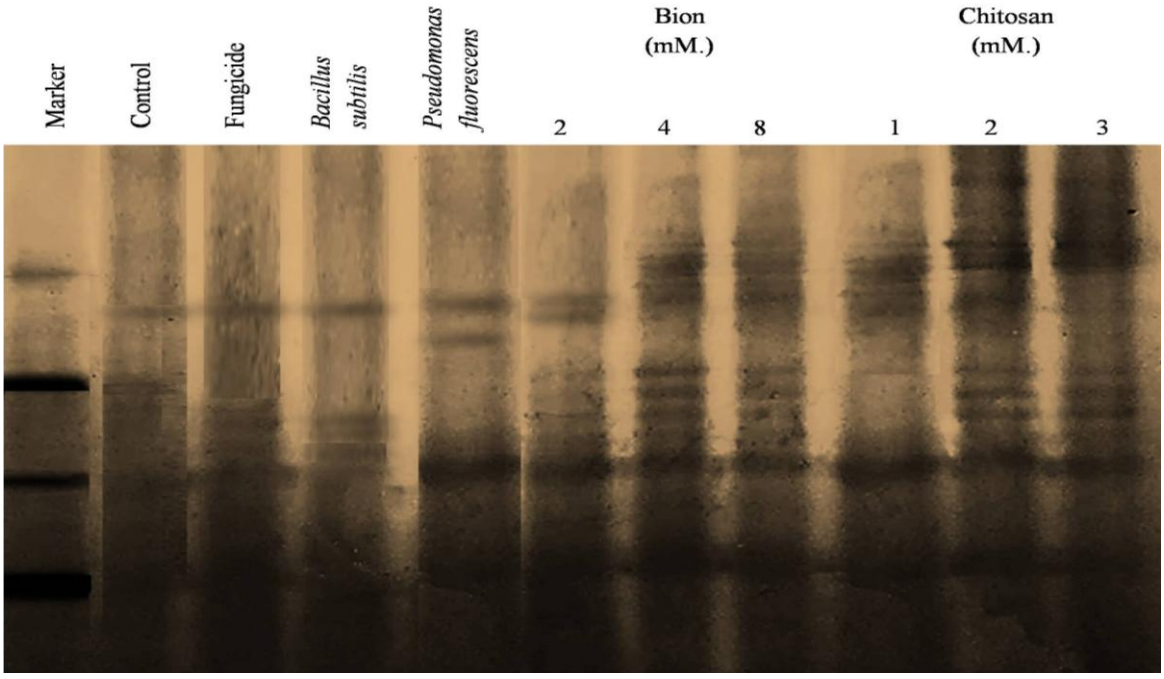

Fig (1): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins extracted from primordial pods growing under artificial inoculation conditions.

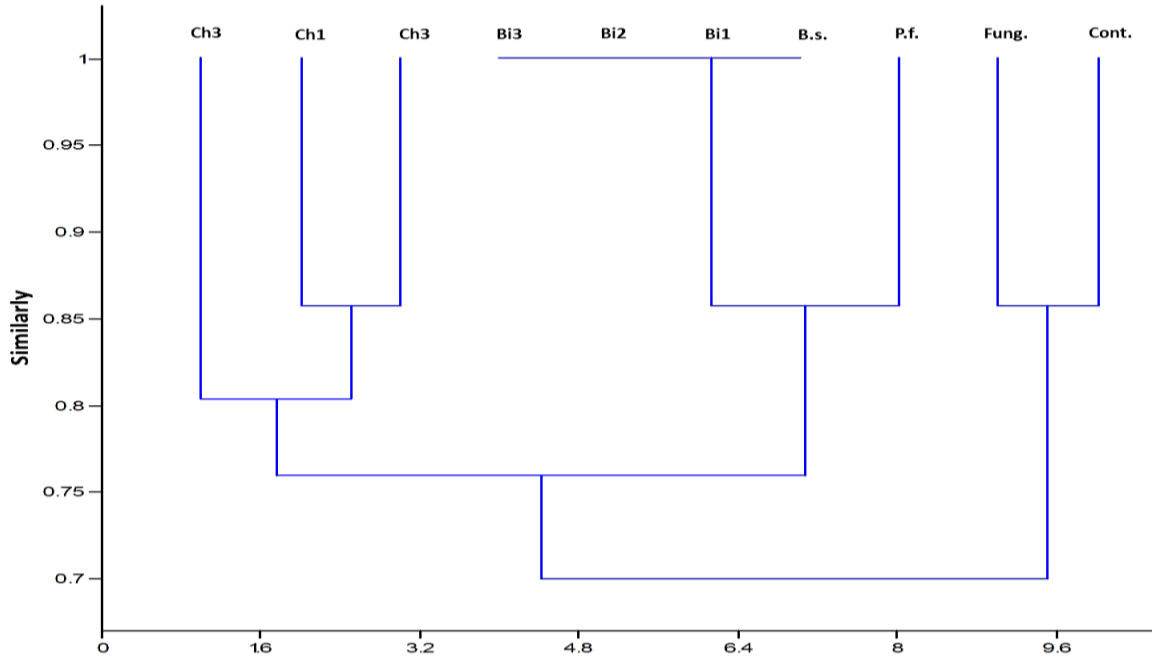

Fig (2): Dendrogram of soluble proteins extracted from peanut primordial pods growing under artificial inoculation conditions for Inducer treatments.

DISCUSSION

Regarding the effect of inducers treatments on the incidence of peanut pod rots diseases and their aflatoxin contamination; the presented data indicate a significant effect of all treatments at their tested concentrations had a significant effect in reducing the incidence of peanut pod rot disease and thus increasing the total pod yield. In this regard chitosan give the highest impact among abiotic inducers while for biotic inducers, *P. fluorescens* was more effect than *B. subtilis*. The efficacy of abiotic inducer was dependent on their concentration which showed clearly relationship between increasing of their concentration and reduced diseases incidence and this already observed in a similar comparison carried out in a different pathosystems by **Aziz *et al.* 2006, Faoro *et al*. 2008 Prakongkha *et al.* 2013, Orzali *et al*. 2017 and Mahmoud *et al*. 2021**.

Meanwhile, the best effect in reducing peanut pod rot and aflatoxin contamination was recorded using chitosan at a concentration of 3 mM. This result is consistent with studies showing that chitosan acts as important elicitors in plants against a wide range of pathogens. Chitosan induces host plants to produce protein, enzymes, and secondary metabolites associated with pathogen defense. This was confirmed in current study, by increases in activities of various classes of PR-proteins **(**appearance of new protein bands) and this was clear in increasing of crude protein % and total amount of amino acid of in treatment associated with chitosan treatments (**Hadwiger, 2013, Xing *et al.*, 2015 and Mahmoud *et al.*, 2021**).

The obtained results revealed that, the phenols content in peanut primordial pods were significantly influenced by chitosan treatment, and the efficacy of chitosan treatment increased with concentration increment, that be confirm the role of chitosan in stimulate the systematic induce resistance in plants (SIR) considers that, phenols one of responses of SIR (**Mahmoud *et al.*, 2021**). Similarly, **Zhang *et al,* 2015**, reported that, the impacts of chitosan on the plant fungal interaction

with *Botrytis cinerea* in tomatoes have been linked with increased of phenolic compounds, elicitation of phytoalexin precursors, and enhanced production of chitinases and other plant defense factors. Moreover, there is a clear relationship between chitosan treatment and increasing of oxidative enzymes activity such as, catalase, peroxidase, and polyphenol odixidase, which are common plant biochemical responses associated with induced resistance (**Zhang *et al,* 2015 and Mahmoud *et al.,* 2021**).This in agreement with many researchers who's reported that, chitosan and its derivatives enhance the activity of chitinase, peroxidase, phenylalanine ammonia-lyase, polyphenol oxidase, superoxide dismutase and catalase in various crops, including wheat, cucumber, tomato, sweet cherries, table grapes, pears, orange, strawberries, and ginseng (**Romanazzi, 2010, Orzali *et al.,* 2017, Xing *et al.,* 2015 and Li *et al.,* 2016**).

The data also revealed that, the application of chitosan significantly increased peanut pod yield, which may be attributed to the role of chitosan in enhancement the growth of plant. This finding aligns with previous studies reporting that chitosan functions as a plant growth promoter in various crops (**Chandrkrachang, 2002 and Chakraborty *et al.,* 2020).**That due to the role of chitosan in reduce stress damage in plant cells by decreasing water content and accelerating several biological macromolecules activities (**Nahar *et al.,* 2012**).

From a different angle, *P. fluorescens* outperformed *B. subtilis* as a biotic inducer for peanut pod rots and aflatoxin contamination, while coming after chitosan treatments in terms of effectiveness. The capacity of *P. fluorescens* to cause systemic resistance (ISR) in plants that phenotypically resembles pathogen-induced systemic acquired resistance (SAR), which has been shown to be effective against bacteria, viruses, and fungi **(Verhagen *et al.,* 2010, and Gruau *et al.,* 2015),** may be the cause of this. Furthermore, *P. fluorescens* causes resistance by means of the SA-dependent SAR pathway, which consists of pathogenesis related (PR) proteins, lipopolysaccharides, siderophores, and salicylic acid (SA) **(Verhagen *et al.,* 2010, and Altinok *et al.,* 2013).**

In conclusion, our experiments based on applications of abiotic and biotic inducers in peanut confirmed that alternatives to the chemical fungicides can contribute to the control of peanut pod rots and aflatoxin contamination. Furthermore, the study demonstrated that, chitosan induces a strong defense response in peanuts when used as-preventive treatment. The effectiveness as a natural, eco-friendly product for controlling peanut pod rots and aflatoxin contamination was clearly established.

Author's contribution

Majority contribution for the whole article belongs to the author(s). The author read and approved the final manuscript.

Competing interests

The author declares that he has no competing interests.

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