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Research Article

The Effect of Using Vitavax Fungicide on Microbial Flora of Peas and Barley Roots and Some Vegetative Characteristics

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Abstract: The fungicide Vitavax was used to fumigate both barley and pea seeds at concentrations; 0.1, 0.3 and 0.5mg/g seeds and kept for a period reaching five months. The data obtained indicate that germination percentage and the plant growth characteristics were slightly affected with differences in the two plants under investigation. With regard to microbial content in both rhizosphere and rhizoplan; the total viable count was slightly affected after one month of storage, but the counts remained at a good rate till the 5th month of storage. The dominant strains belonged to *Bacillus, Pseudomonas*, *Micrococcus*, *Sarcina* and *Actinomyces.* The counts of *Azotobacter* were not affected in a high degree. The fungicide concentration of 0.3mg/g was the best among the treatments with 3 months storage period, for either plant growth parameters or bacterial counts although it reached five months with other treatments.

Keywords: Vitavax, Rhizosphere, Rhizoplan, Growth parameters of peas and barley, Dominant and resistant isolates.

1. Introduction

Fungicides are designed to suppress the biochemical and physiological metabolism of the target phytopathogenic fungi, at the same time might have lasting effects on non-target soil-inhabiting microorganisms (Tu, 1993). A lot of research work has been conducted for assessing the dissipation of fungicides in soil and their effects on soil microorganisms (Martinez-Toledo *et al.*, 1998; Smith *et al.*, 2000; Sigler and Turco, 2002; Kinney *et al.*, 2005 and Bending *et al.*, 2007). The application of fungicides to the soil to control plant diseases has become a common practice in crop production in many parts of the world. However, knowledge of the possible environmental hazards posed by the use of such xenobiotics is much more readily available for aquatic ecosystems than terrestrial ecosystems and, because of this, there is a need to characterize the most suitable and susceptible biological indicators of adverse effects of fungicides on the soil environment (Peichl and Reiml, 1990). Seeds are considered to be suitable as a host to maintain the pathogenic microorganisms even in the absence of the host. Treating such seeds with fungi- or bactericides will protect them from being attacked by fungi, nematodes or other pests (Buss *et al.*, 2001). Treating vegetable and crop seeds with fungicides will protect them against soil-borne fungi which could cause diseases, especially root-rot (Pimentel and Greiner, 1997). With the wide increase of the use of such chemicals, it was found that they have harmful effects on humans, animals, plants and microorganisms. Therefore, there was a crucial need to study their toxicity (Goldstein *et al.*, 1985 and Ramadan *et al.*, 1990). A portion of the pesticides and fungicides interact with microorganisms in the soil and rhizosphere (Wooton *et al.*, 1993). In their work on the fungicide Thirm, Sahin and Tamer (2000) reported that thiram-degrading fungi were identified as *Aspergillus niger, A. flavus* and *Penicillium steckii.* They also added that bacterial isolates were assigned to the genera; *Bacillus, Arthrobacter, Moraxella*-like,

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Acinetobacter and *Streptomyces*. Soil fungi and actinomycetes are not susceptible to herbicides and insecticides as they are to fungicides (Anderson, 1978). Quite a small number of researches focused on the use of Vitavax in recent years (Digrak and Ozcelik, 1998; Maher *et al.*, 2005 and Abdel-Aziz, 2006).

From this point, the study aims to investigate the effect of fumigating barley and pea seeds with Vitavax (Carboxin) on germination, vegetative characters and on some rhizosphere and rhizoplane microorganisms.

2. Materials and Methods

2.1 Seeds

Seeds of both peas *Pisum sativum* and barley *Hordeum vulgare* L. (Rayhan variety) were collected from Tsawa Station for developed Seeds. Seeds were carefully selected with no apparent infections.

2.2 Fungicide

The Vitavax-PCNB fungicide (1,4-Oxathiin-3-carboxamide, 5,6-dihydro-2-methyl-N-phenyl-) was also collected from Tsawa Station for developed Seeds. The fungicide was used to coat the seeds with the following concentrations; 0.1mg/ g, 0.3mg/g seeds and 0.5mg/g seeds. Then seeds were stored at room temperature in material bags (3 bags for each concentration) for one, 3 and 5 months.

2.3 Germination

Twenty seeds were placed in a petri dish on a filter paper then kept always wet by adding tab-water. The germination percentage was calculated with each group of seeds after being stored for 1, 3 and 5 months.

2.4 Cultivation

A pot experiment containing the normal sandy loamy soil used in normal fields was then carried out, three pots for each treatment with the two tested plants in a randomized complete design (RCD). Pots were irrigated two days interval. All plants were fertilized with the recommended N fertilizer (180 kg N/ha) as Urea (46% N) and 100 kg/ha Potassium sulfate, for barley. For peas, superphosphate 15.5% (230 kg/ha), ammonium sulfate 20.5% (230 kg/ha) and potassium sulfate 48% (115 kg/ha) were used. Untreated seeds were used either for germination and cultivation as a control.

2.5 Determinations

2.5.1 Plant growth parameters: Samples were taken after 45 days of cultivation. Root length; shoot height, plant height, number of leaves, fresh weight and dry weight as an average of 5 plants for each treatment for both plants. Number of tillers was taken only for barley.

2.5.2 Microbiological determinations: Soil samples from both rhizosphere and rhizoplan were analyzed after 45 days to enumerate the colony forming units (CFU) of total viable bacteria and free nitrogen fixing bacteria by means of the serial dilution technique and pour plate method (Salle, 1973). Analyses were performed in triplicate. The agar plates were incubated at 28 ± 2^0C for 48 h. The colonies, which grow on agar, were counted and the average was taken as the total bacterial density of the soil sample. Aliquots (1.0ml) of the serially diluted soil samples were also plated on sterile Ashby's mannitol-sulfate agar. The medium contained per liter of double distilled water: KH_2PO_4 (0.2g), $MgSO_4.7H_2O$ (0.2g), NaCl (0.2g), $CaSO_4$ (0.1g), $CaCO_3$ (5.0g), Mannitol (10.0g) and agar (15.0g). The plates were incubated at 28^0C for 10 days. The colonies grown were taken as free-living nitrogen fixing bacteria.

2.5.3 Isolation and identification: Bacterial colonies grown well on the plates were isolated, purified and identified, to the genus level, after microscopic examination following Bergey's Manual of Systematic Bacteriology (1994).

2.6 Statistical Analysis

The obtained data were subjected to analysis of variance as described by Gomez and Gomez (1984).

Table 1. The effect of fumigation with Vitavax at different concentrations and storage period on germination percentage of both barley and pea seed.

Plant	Storage period (month)	Concentrations (mg/g)			
		Control	0.1	0.3	0.5
		Germination percentage			
Barley	1	100.00	88.85	100	81.25
	3	100.00	86.50	75.00	43.75
	5	100.00	56.25	68.75	43.75
Peas	1	100.00	62.50	100.00	68.75
	3	100.00	43.75	100.00	56.25
	5	100.00	37.50	81.25	50.00

LSD for storage period; 1.074 and 0.738 at 0.01 and 0.05 levels, respectively.
LSD for concentrations; 1.621 and 1.204 at 0.01 and 0.05 levels, respectively.

3. Result and Discussion

3.1 Germination percentage

Data in Table (1) presents the germination percentage of both peas and barley and the effect of storage period after fumigation. It is noticed that barley gave 100% germination after a month of storage then decreased to 75.0 and 68.75% after 3 and 5 months of storage, respectively when using the treatment 0.3mg/g. Where the percentage reached 88.85 and 81.25% after the same period (one month), when using 0.1 and 0.5mg/g, respectively, then sharply decreased when 0.5mg/g concentration was used after 3 and 5 months of storage. This indicates that the recommended dose is better after one month of storage, but 0.1mg/g could be recommended for 3 months storage. This also could be explained on the basis that some fungicides could affect the embryo of some seeds as was previously mentioned by Saeidi and Mirik (2006) in their study on flax-seed treated with Captan 0.2% and Carbendazim 0.15%, who reported that seed germination was not significantly affected except for some seeds after long storage periods. For peas, the germination percentage showed the same trend where it was better after 1 and 3 months (100% with 0.3mg/g Vitavax) than after 5 months. Where the highest germination percentages with the two other concentrations were 62.5 and 68.75% after one month of storage the dramatically decreased. It was noticed that all used concentrations had significant effects on germination and that the recommended dose is the best although the germination percentage decreased with the elongation of storage period.

3.2 Effect of fumigation and storage on growth parameters

Data in Table (2) points to the effect of fumigation and storage on growth parameters of barley plants. Regarding root length, the best results were obtained with plants from seeds fumigated with 0.3 and 0.5mg/g after 3 months of storage superior to the control and 0.1mg/g. As for shoot length, the best results were obtained with plants from seeds fumigated with 0.3mg/g after 3 months of storage although the results were near from the two other concentrations regarding plant height with 0.3 and 0.5mg/g with high significance with the three month period of storage. The number of leaves at all concentrations, with a slight increase at 0.03mg/g, showed no significant difference. As for the number of tillers, the best results were obtained with 0.5mg/g concentration which continued to the fifth month of storage. For the fresh weight, 0.3mg/g concentration surpassed the other concentrations, where the dry weight showed that both 0.3 and 0.5mg/g were close to each other although 0.3mg/g Vitavax concentration could be recommended for treating seeds to 3 month period of storage.

Table 2. Effect of fungicide concentration and storage period on growth parameters of barley.

Character	Storage period (month)	Concentration (mg/g)			
		Control	0.1	0.3	0.5
Root length (cm)	1	6.0	5.0	8.0	8.0
	3	6.8	7.5	9.0	9.5
	5	7.2	6.7	8.2	9.0
LSD for concentrations; 0.96 and 0.70 at 0.01 and 0.05, respectively					
Shoot height (cm)	1	16.00	18.00	20.00	19.50
	3	22.53	24.50	25.00	24.50
	5	23.00	23.10	20.00	22.00
LSD for concentrations; 1.49 and 1.09 at 0.01 and 0.05, respectively					
Plant height (cm)	1	22.00	23.00	28.00	27.50
	3	29.33	32.00	34.00	34.00
	5	30.20	29.80	28.20	31.00
LSD for concentrations; 2.02 and 1.47 at 0.01 and 0.05, respectively					
Number of leaves	1	3.8	4.0	6.0	4.0
	3	4.2	5.0	5.5	5.0
	5	3.6	5.0	4.0	4.0
LSD for concentrations; 0.80 and 0.58 at 0.01 and 0.05, respectively					
Number of tillers	1	2.9	2.0	4.0	3.0
	3	3.7	4.0	3.0	4.0
	5	3.2	4.0	3.0	4.0
LSD for concentrations 0.70 and 0.51 at 0.01 and 0.05, respectively					
Fresh weight (g)	1	1.95	1.10	2.90	1.60
	3	1.80	1.90	2.60	1.90
	5	1.55	1.80	2.10	1.70
LSD for concentrations; 0.40 and 0.30 at 0.01 and 0.05, respectively					
Dry weight (g)	1	0.36	0.40	0.76	0.50
	3	0.60	0.50	0.70	0.70
	5	0.37	0.35	0.40	0.35
LSD for concentrations; 0.13 and 0.07 at 0.01 and 0.05, respectively					

Table 3. Effect of fungicide concentration and storage period on growth parameters of pea plants.

Character	Storage period (month)	Concentration (mg/g)			
		Control	0.1	0.3	0.5
Root length (cm)	1	7.0	8.0	9.0	9.5
	3	8.1	8.2	8.7	9.0
	5	9.0	8.2	9.0	9.5
LSD for concentrations; 0.96 and 0.70 at 0.01 and 0.05, respectively					
Shoot height (cm)	1	14.5	14.0	15.0	13.0
	3	11.5	13.7	13.0	13.5
	5	11.9	13.8	15.0	13.0
LSD for concentrations; 1.49 and 1.09 at 0.01 and 0.05, respectively					
Plant height (cm)	1	21.5	22.0	24.0	22.5
	3	19.6	21.9	21.7	22.5
	5	20.9	22.0	24.0	22.5
LSD for concentrations; 2.02 and 1.47 at 0.01 and 0.05, respectively					
Number of leaves	1	18.0	21.0	22.0	21.0
	3	20.0	21.0	22.0	21.0
	5	21.0	21.0	22.0	20.0
LSD for concentrations; 0.80 and 0.58 at 0.01 and 0.05, respectively					
Fresh weight (g)	1	2.60	2.00	2.70	2.30
	3	3.10	2.70	3.20	3.20
	5	2.76	2.80	2.90	2.80
LSD for concentrations; 0.40 and 0.30 at 0.01 and 0.05, respectively					
Dry weight (g)	1	0.35	0.50	0.80	0.40
	3	0.32	0.43	0.35	0.30
	5	0.52	0.32	0.60	0.45
LSD for concentrations; 0.13 and 0.07 at 0.01 and 0.05, respectively					

As for the peas, Table (3) indicates that 0.5mg/g concentration gave the highest root length till a storage period of 5 months, while for shoot length 0.3mg/g was superior till the fifth month of storage.

It was clear that 0.3mg/g concentration was the best regarding the dry weight. The best two storage periods were after 1 and 5 months. Collectively, for peas both 0.3 and 0.5 mg/g concentrations are the best for peas till a period of 5 months storage. The results are in agreement with the findings of Karanth and Vasantharajan (1979) that used Vitavax and Dexon to coat barley and wheat seeds, where they noticed that the two plants were not affected much in vegetative stage but the yield decreased by 10% compared to the control.

3.3 Effect of storage period and different Vitavax concentration on TVC

The soil microbial community involves a complex interwoven relationship between organisms of different trophic levels. Some microbial groups are able to use an applied pesticide or fungicide as a source of energy and nutrients to multiply, whereas the pesticide or fungicide could be toxic to other organisms (Johnsen *et al*., 2001). Data in Table (4) shows the TVC for the two tested plants in the rhizosphere. Regarding barley, the counts haven't been affected when 0.3mg/g was used during all storage periods. The best results were obtained after 3 months followed by 1 and 5 months (8.5, 6.7 and 6.4 x 10^5), respectively followed by 0.1mg/g where the best results were obtained after 1 month of storage followed by 3 and 5 months (5.9, 5.1 and 3.6 x 10^5), respectively to be in an agreement with the findings of Bassio *et al*., (1998). For peas, the best results obtained were at 0.5mg/g followed by 0.3 and 0.1mg/g, respectively, especially after being stored for three months (9.0, 8.4 & 6.9 x 10^5) which were better than the control. The counts remained also remained high for 0.3 and 0.5 mg/g till the 5th month of storage.

Regarding the dominant genera which could be considered resistant to the Vitavax concentrations used; for barley, they were *Bacillus*, *Pseudomonas*, *Micrococcus* and *Actinomyces* for three months of storage where both *Bacillus* and *Pseudomonas* were found dominant to the 5th month of storage. This could be explained by the resistance of *Bacillus* and that some *Pseudomonas* members are able to use the fungicide as carbon source. As for the peas, the situation did not differ a lot where the dominant genera were *Bacillus*, *Pseudomonas*, *Micrococcus* and *Actinomyces* till the 3rd month of storage where the genera of *Bacillus, Pseudomonas & Micrococcus* remained till the 5th month of storage to agree with the above mentioned explanation which was also reported by Leiss (2004).

In the rhizoplan area, Table (5) indicates the changes in TVC with the barley plant where the best results were obtained with the plants where seeds were kept for 3 months. For the concentrations; 0.1mg/g gave the highest counts followed by 0.3 and 0.5mg/g, respectively (11.6, 7.8 and 7.2 x 10^5). With regard to peas, the best results were obtained with 0.1mg/g concentration followed by 0.3 and 0.5mg/g at storage period after 3 months (13.0, 9.8 and 9.0 x 10^5, respectively).

Table 4. Effect of storage period after coating with Vitavax on the changes in TVC (cfu/g) in the rhizosphere.

Plant type	Storage period (months)	Concentrations (mg/g)			
		Control	0.1	0.3	0.5
		TVC x 10^5			
Barley	1	2.06	5.90	6.70	4.00
	3	3.11	5.10	8.50	3.70
	5	3.06	3.60	6.40	3.30
Peas	1	3.73	7.10	4.30	5.40
	3	3.90	6.90	8.40	9.00
	5	4.21	5.40	6.90	7.70
LSD for concentrations; 0.44 and 0.33 at 0.01 and 0.05, respectively					

Table 5. Effect of storage period after coating with Vitavax on the changes in TVC (cfu/g) in the rhizoplan.

Plant type	Storage period (months)	Concentrations (mg/g)			
		Control	0.1	0.3	0.5
		TVC x 10^5			
Barley	1	3.50	5.80	3.90	1.50
	3	6.60	11.60	7.20	7.80
	5	5.70	6.40	6.50	4.50
Peas	1	3.16	5.30	4.60	7.30
	3	4.65	13.00	9.80	9.00
	5	4.11	3.40	6.70	5.90
LSD for concentrations; 0.44 and 0.33 at 0.01 and 0.05, respectively					

Table 6. Effect of storage period after coating with Vitavax on the changes in *Azotobacter* counts (cfu/g) in the rhizosphere.

Plant type	Storage period (months)	Concentrations (mg/g)			
		Control	0.1	0.3	0.5
		TC of *Azotobacter* x 10^5			
Barley	1	8.10	13.40	13.10	8.80
	3	8.60	14.00	17.60	7.00
	5	8.40	13.90	14.50	4.90
Peas	1	6.40	8.40	13.20	8.20
	3	7.20	10.10	17.10	9.70
	5	6.80	6.40	17.40	6.60
LSD for concentrations; 0.53 and 0.39 at 0.01 and 0.05, respectively					

3.4 Changes in the total count of *Azotobacter* in the rhizosphere and rhizoplan

Table (6) shows the effect of coating seeds with Vitavax and storage periods on the total count of *Azotobacter* in the rhizosphere of the two tested plants. For barley, 0.3mg/g concentration gave the highest counts followed by 0.1mg/g (17.6 and 14.0 x 10^5, respectively), after three months of storage superior to the control and 0.5mg/g concentration. After 5 months, there was a slight decrease for 0.1 and 0.3mg/g concentration while the decrease was very sharp for the 0.5mg/g one. As for the peas, the best results were obtained with the 0.3mg/g concentration even after 5 months of storage which indicates that the effect of this fungicide on this type of microbes very weak.

Regarding the effect of storage period and the fungicide concentration on the counts of *Azotobacter* in the rhizoplan, data in Table (7) indicate that the counts were dramatically affected although the 0.1mg/g concentration was the best till 3 months of storage with barley but lower than those in the rhizosphere. With peas after 5 months of storage, the counts gave the highest level with 0.1mg/g followed by 0.3 and 0.5mg/g, respectively with very narrow difference than the control. This could be explained that the amount of the fungicide residue may be still a bit higher in this area which consequently affected the counts. This agrees with the observation of Finkelstein and Golovelva (1988) who indicated that low concentrations of pesticides and fungicides stimulated the growth of nitrogen fixing bacteria whereas higher concentrations inhibited the nitrification process.

These results are in agreement with what was reported by Hata *et al.*, (1986) and Topp *et al.*, (2000) and highly agree with the findings of Druska (2003), but on the contrary with what was mentioned by Stefaniak *et al.*, (1993a & b) who mentioned that *Azotobacter* and other free living nitrogen fixing bacteria were capable of resisting the effect of many fungicides applied and they also reported that this could be due to their ability, especially for *Azotobacter* with the presence of capsule around the cells, to protect themselves against the fungicidal effect or their ability to degrade these fungicides to non-toxic substances.

Table 7. Effect of storage period after coating with Vitavax on the changes in *Azotobacter* counts (cfu/g) in the rhizoplan.

Plant type	Storage period (months)	Concentrations (mg/g)			
		Control	0.1	0.3	0.5
		TC of *Azotobacter* x 10^5			
Barley	1	5.30	4.60	3.20	4.40
	3	6.80	13.00	9.00	6.60
	5	6.40	6.30	6.20	4.30
Peas	1	4.10	4.60	4.10	3.00
	3	4.30	4.70	4.93	5.00
	5	4.42	8.10	6.60	3.80
LSD for concentrations; 0.44 and 0.33 at 0.01 and 0.05, respectively					

4. Conclusion

The use of the antifungal Vitavax in low or high doses to cover the seed for protection from infection and long-term preservation of seeds proved to have some effects on soil microflora and on some growth parameters, in turn. The use of recommended dose proved to have little or no effect on the studied characteristics. It's highly recommended to use either the recommended dose or a bit higher dose that will preserve seeds and will not affect either the studied microbial flora or even growth characteristics.

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