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EFFECT OF SEED BORNE MYCOFLORA ON SEEDLING VIGOUR OF PEA VARIETIES AND ITS MANAGEMENT BY SEED PRIMING

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ABSTRACT

The present study was conducted at Department of Plant Pathology, IGKV, Raipur (CG) during 2017-18. Seed health is affected by various factors, among which the most important is seed borne fungi that cause reduction in seed germination and seedling vigour. Seven pea varieties *viz*. IPFD 10-12, Paras, Indira matar, KPMR 400, Shubhra, Ambika and local variety from randomly selected village were taken to test the effect of isolated seed borne fungi on seedling vigour under seedling symptoms in pot culture condition by using seed and soil inoculation technique. Most of the seed borne mycoflora reduced the seedling vigour index and some were not. Two seed borne mycoflora of pea seeds i.e. *Rhizopus* sp. and *Fusarium* sp. were found pathogenic and seed to plant transmissible in nature whereas increased seedling vigour of pea varieties were recorded due to growth promoting ability of *Trichoderma* sp. Seed lots of pea varieties were also treated with fungicides and biocontrol agent (*Trichoderma* + Vermicompost) and observed increased seedling vigour index as compared to untreated ones. It is summarized that seed borne mycoflora reduced the seedling vigour index whereas, fungicidal and biocontrol agent *Trichoderma*+ Vermicompost increased the seedling vigour index by keeping seed borne mycoflora under check.

(Key words : Pea, seed borne mycoflora, seedling vigour index, seed priming)

INTRODUCTION

Pea is cultivated in temperate regions at high elevations or during cool season in warm regions throughout the world. It is originated from Mediterranean Region of Southern Europe and Western Asia. Pea is the third most important pulse crop at global level, after dry bean and chickpea and third most popular *Rabi* pulse of India after chickpea and lentil. It provides a variety of vegetarian diet hence, liked throughout the world. One of the major constraints in the pea production is the attack of various diseases at different stages of growth including seed-borne disease. Germination, growth and productivity of crop plants are affected by the infection of various mycoflora. A seed-borne pathogen present externally or internally or associated with the seed as contaminant may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in the development of disease at later stages of plant growth of systemic or local infection.

Field fungus associated with seeds cause deterioration of seed quality, affect viability and reduces germination (Srivastava and Gupta, 1981). A large number of mycoflora was reported to be associated with the pea seeds like *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Fusarium* sp., *Chaetomium* sp., *Curvualria* sp., *Penicillium* sp., *Rhizoctonia* sp., *Rhizopus* sp. etc. causes reduction in yield of crop. In view of above, present study was conducted to know the effect of seed borne mycoflora on seedling vigour index and its management by seed priming.

MATERIALS AND METHODS

1. Effect of seed borne mycoflora on seedling vigour of pea varieties (seed inoculation technique)

Apparently healthy surface sterilized (1.0 % NaOCl) seeds were taken for this study. The seeds were rolled on 7-10 days old sporulating culture of individual mycoflora thriving on PDA in Petri plate. The rolled seeds were grown in pots filled with sterilized soil. A set of control was also kept with surface sterilized seeds sown in sterilized soil in pots. The seedling growth was assessed in term of seedling vigour index 21 days after sowing. The germination percentage, root length and shoot length were recorded to calculate seedling vigour index of each inoculated seed samples. The shoot length measured from the base of the shoot to upper most leaf tip. For measuring the root length, plant was carefully uprooted first, gently washed and carefully placed on clean transparent glass piece. The length of root system was measured from collar region to the end of longest tip. The seedling vigour index was calculated by

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using the following formula given by Abdul- Baki and Anderson (1973).

Seedling vigour index = (Mean shoot length + Mean root length) x Germination percentage.

2. Effect of seed borne mycoflora on seedling vigour of pea varieties (soil inoculation technique)

All seed borne fungi detected in various methods were grown separately and multiplied in sterilized wheat grain substrate. Substrate was inoculated with seven days old culture of individual fungus separately. The inoculated substrate was incubated at 25 ± 2 ^{0}C for ten days. Substrates were shaken every day to avoid clumping. Pots were filled with sterilized soil and inoculated by each mycoflora inoculum. For soil inoculation, upper four cm layer of the soil was thoroughly mixed with culture grown in wheat medium @ 10 g pot^{-1} and watered to just wet the inoculated. Pots were kept in glass house for 72 hours for proper soil infection and establishment of mycoflora before sowing of pea seeds. Seeds were surface sterilized (1.0 % NaOCl) before sowing followed by three washing with sterile distilled water and were sown in inoculated soil @ 20 seeds pot^{-1}. A set of control was also kept with surface sterilized seeds sown in sterilized un-inoculated soil. Pots were watered at regular intervals. The seedling growth was assessed in term of seedling vigour index 21 days after sowing as described earlier.

3. Effect of seed priming for the management of seed borne mycoflora of pea varieties

To find out the effect of seed priming on seedling vigour, six seed dressing fungicide *viz.,* Blitox 50 (Copper Oxychloride), SAAF (Carbendazim + Mancozeb), Avtar (Hexaconazole + Zineb), Bavistin (Carbendazim), Octave (Propineb) and bio agent (*Trichoderma* + Vermicompost) were taken at their recommended dose along with a control (without treated). Treated and untreated seeds were grown in pots filled with sterilized soil. The seedling growth was assessed in terms of seedling vigour index 21 days after sowing. The germination percentage, root length and shoot length were recorded as described above to calculate seedling vigour index of each treatment and seed sample.

RESULTS AND DISCUSSION

1. Effect of seed borne mycoflora on seedling vigour of pea varieties (seed inoculation technique)

It is depicted from data presented in table 1(a) and (b), that seedling vigour was markedly reduced by some of the seed borne mycoflora when evaluated by seed inoculation technique. Overall impact in reducing seedling vigour index was shown by *Rhizopus* sp. across all seven varieties evaluated as compared to that of control.

Maximum reduction in seedling vigour index of IPFD 10-12 variety was caused by *Rhizopus* sp. (94.34%) followed by *Curvularia* sp. (89.54%) and *Chaetomium* sp. (87.71%). *Rhizopus* sp. caused maximum reduction in seedling vigour index of seed lot of Indira matar, Shubhra, Ambika and local variety (99.31%, 96.32%, 99.78% and 87.52%, respectively) followed by *Fusarium* sp. 97.12%, 95.96%, 91.76% and 86.39%, respectively.

Rhizopus sp. (94.57%) showed maximum reduction in seedling vigour index of pea varieties as compared to that of control followed by *Fusarium* sp. (91.27%). Minimum reduction in seedling vigour index of all pea varieties were recorded in seed inoculation with *Penicillium* sp. (11.62%) followed by *Aspergillus flavus* (52.63%).

Overall, seedling vigour of pea varieties by *Trichoderma* sp. inoculation recorded as 133.50%. Seeds of pea varieties inoculated with *Trichoderma* sp. showed reverse trend among all mycoflora. *Trichoderma* sp. may exhibits plant growth promoting activity, hence it increased seedling vigour of pea varieties as compared to that of control.

Reduction in seedling vigour may be attributed due to inhibition of germination of seed by inoculated mycoflora, some of the weak seedlings with light green colour leaves and reduced plumule and radical length or wilt like symptom and rotting of roots of seedlings were also noticed. The under developed symptom shown by some of the seedling were subjected to isolation of mycoflora from infected plant / root tissues. Isolation from wilted type plant tissue yielded the fungus identical to with the *Fusarium* sp. which was isolated from seed sample. Similarly, isolation from infected root tissues yielded the fungus identical with the *Rhizopus* sp. which was isolated from seed samples.

Hence, it was proved that the detected seed borne mycoflora namely *Rhizopus* sp. and *Fusarium* sp. were pathogenic to pea seeds and observed seed transmissible in the present study. Hirwani (2016) and Wani and Aalum (2018) also observed reduction in germination and plant vigour index in pea seeds due to various mycoflora. Pradhan (2017) also recorded that overall impact of *Rhizopus* sp. and *Fusarium* sp. in seedling vigour of five varieties of mungbean. Seed borne mycoflora were found to reduce the seed germination, root and shoot length significantly in various legumes also. Chaudhary *et al*. (2017) reported seed borne mycoflora and their culture filtrate caused considered reduction in germination per cent and seedling growth as compared to untreated check in pigeopea. Pradhan (2017) observed that the seedling vigour of mung bean was markedly reduced by some of the seed borne mycoflora. In seed inoculation technique, overall impact in reducing seedling vigour index was shown by *Rhizopus* sp. across all five seed lots evaluated as compared to that of control. Findings of all above mentioned workers supports the findings of present study.

2. Effect of seed borne mycoflora on seedling vigour of pea varieties (soil inoculation technique)

Soil inoculation technique was used to know the effect of seed borne mycoflora on seedling vigour index and data are presented in table 2(a) and (b). It was clear from the table that *Fusarium* sp. reduced the vigour index

maximum i.e. 92.12% irrespective of seed lots followed by *Rhizopus* sp. (88.62%) and *Chaetomium* sp. (82.46%) in comparison to control. Minimum reduction in seedling vigour index of pea varieties were recorded in *Penicillium* sp. (10.06%) followed by *Aspergillus flavus* (48.11%).

In case of *Trichoderma* sp. increased seedling vigour (120.71%) of pea varieties as compared to control was observed, while decreased seedling vigour as compared to control was observed in soil inoculated with other mycoflora.

Pradhan (2017) conducted a pot experiment and reported that maximum seedling vigour index was recorded in Raigarh district seed lot in C.G. Triocap (2900.79%) treatment which was 67.24 per cent more than that of control followed by Jagdalpur district in Devithiram (2716.66%) which was 56.44 per cent more than that of control. Least vigour index was recorded in Kawardha district (898.32%) in control (untreated). Maximum mean increase in vigour index over control (56.61%) was recorded in Mancozeb across the five seed lots treated followed by *Trichoderma* treated seeds of five seed lots (50.19%).

Many plant pathogenic fungi are known to produce phytotoxic metabolites (Vidhyasekaran *et al.,* 1970). Importance of the production of such toxic metabolites was more obvious when the pathogen was seed borne because it may either inhibit seed germination or adversely affects the initial growth of seedlings. The reduction in germination due to seed borne mycoflora may be attributed to the production of aflatoxin in good grains interferes with protein synthesis by inhibiting the incorporation of amino acid into protein resulting in non-germination of embryo. Aflatoxin affects the plants by inhibitions of seed germination, elongation of hypocotyl or root of developing seeds.

Wani and Aalum (2018) observed that the germination and seedling vigour of pea varieties were most affected by *Fusarium* sp. Decreased plumule and radicle length were recorded maximum in case of soil inoculated with culture of *Fusarium* sp. as compared to control. They observed that root growth and shoot growth in *Pisum sativum* (L.) was sensitive to culture filtrate of pathogenic fungi. All filtrates reduced root length and shoot length with variable phytotoxic potential significantly. The seedling vigour was less affected by *Aspergillus flavus* and *Penicillium* sp. In the present study, seedling vigour was less affected by *Penicillium* sp. and *Aspergillus flavus* confers the above findings.

3. Effect of seed priming for the management of seed borne mycoflora of pea varieties

In glass house condition, pot experiment was conducted to know the effect of seed priming by different fungicides and bio control agent (*Trichoderma* + Vermicompost) on the seedling vigour of different varieties of pea. Fungicide and *Trichoderma* + Vermicompost treated and untreated seeds were sown in pre sterilized soil and vigour index was recorded 21 days after sowing. Data presented in table 3(a) and (b) shows that all the fungicides and *Trichoderma* + Vermicompost treated seeds had higher vigour index as compared to that of control.

Maximum seedling vigour index was recorded from Indira matar seed lot in *Trichoderma* + vermicompost treatment (5608) which was 174.90 per cent more than that of control followed by Ambika in SAAF treatment (4871) which was 102.36 per cent more than that of control. Least vigour index was observed in local variety in treatment with AVTAR (1048) and percentage increase over control in local variety was 44.79%. The above result clearly showed that fungicides and bio control agent *Trichoderma* taken in the study could be able to reduce the mycoflora associated with seeds and thereby increase the vigour index and in untreated seeds vigour index was less than the treated seeds.

Analysis of data presented in table 3(b) reveals that maximum mean increase over control were recorded in *Trichoderma* + Vermicompost (134.1%) across the seven seed lots treated. This might be attributed that *Trichoderma* + Vermicompost not only reduces seed borne mycoflora but also exhibit the plant growth promoting activity and thereby higher vigour index was recorded over control across the seven seed lots of pea tested. SAAF treated seeds of seven seed lots of pea recorded second highest mean increase over control (89.86%) and least mean increase over control were recorded in AVTAR (52.74%).

It is clear from the above finding that seed borne mycoflora reduced the seedling vigour index whereas, fungicidal and biocontrol agent *Trichoderma*+ Vermicompost increased the seedling vigour index by keeping seed borne mycoflora under check. Devmani *et al*. (2017) found that out of seven fungicides used for seed treatment of munbean, seed treatment with captan @ 4 g $kg^{-1}$of seed significantly reduced seed mycoflora (78.68 %). Seed treatment with *Trichoderma harzianum* @ of 8 g kg^{-1} of seed reduced the seed borne mycoflora up to (69.63 %) followed by *Pseudomonas fluorescences* (66.49 %) and *Trichoderma viride* (64.39 %). Singh *et al.* (2017) evaluated the effect of seed priming treatments on seed quality of field pea (*Pisum sativum* L.) on pea variety KPMR-522 (Jay). Investigation revealed that seed priming with sodium molybdate @ 500 ppm + seed coating with *T. harzianum* @ 15 g Kg^{-1} seed was significantly improved the seed quality of harvested seeds with per cent improvement of 5.75 and 8.00 % in germination, 15.37 and13.73 % in shoot length, 17.03 and 16.05% in seedling dry weight, 13.06 and 17.96 % in seed vigour index. The above findings support the findings of present investigation.

Table 1(a). Effect of seed borne mycoflora on seedling vigour of pea varieties (seed inoculation technique)

Mycoflora	Seedling vigour index						
	IPFD 10-12	Paras	KPMR 400	Indira matar	Shubhra	Ambika	Local variety
A.flavus	249.20	1202.30	1301.40	931.40	894.00	1037.20	324.50
A.niger	143.60	922.50	1161.60	876.10	911.00	986.30	211.70
Alternaria sp.	198.90	261.00	802.70	437.80	164.20	347.50	103.60
Chaetomium sp.	118.40	351.20	786.40	338.50	150.20	417.90	120.00
Trichoderma sp.	2715.70	3863.60	4800.00	5591.00	4025.00	4560.80	1915.70
Penicillium sp.	859.20	1242.00	2945.20	1856.50	1898.00	1728.10	686.40
Curvularia sp.	100.70	515.20	1020.30	135.80	113.40	385.50	110.50
Fusarium sp	125.30	255.00	0	57.50	97.40	197.40	97.40
Rhizopus sp.	54.50	196.30	10.60	13.80	88.70	5.05	89.30
Control	963.50	1319.50	3067.00	2001.80	2415.10	2398.20	715.90

Table 1(b). Effect of seed borne mycoflora on seedling vigour of pea varieties (seed inoculation technique) (% increase or decrease over control)

Mycoflora	% increase (+) or decrease (-) over control							Mean increase/ decrease over control
	IPFD 10-12	Paras	KPMR 400	Indira matar	Shubhra	Ambika	Local variety	
A.flavus	-74.00	-8.88	-57.56	-53.47	-62.98	-56.75	-54.67	-52.63
A.niger	-85.09	-30.08	-62.12	-56.23	-62.27	-58.87	-70.42	-60.72
Alternaria sp.	-79.73	-80.21	-73.82	-78.12	-93.20	-85.50	-85.52	-82.30
Chaetomium sp.	-87.71	-73.38	-74.35	-83.09	-93.78	-82.57	-83.23	-82.58
Trichoderma sp.	181.85	192.80	56.50	179.29	66.65	90.17	167.59	133.50
Penicillium sp.	-10.82	-5.87	-3.97	-7.25	-21.41	-27.94	-4.12	-11.62
Curvularia sp.	-89.54	-60.95	-66.73	-93.21	-95.30	-83.92	-84.56	-82.03
Fusarium sp	-86.99	-80.67	-100.00	-97.12	-95.96	-91.76	-86.39	-91.27
Rhizopus sp.	-94.34	-85.12	-99.65	-99.31	-96.32	-99.78	-87.52	-94.57

Table 2(a). Effect of seed borne mycoflora on seedling vigour of pea varieties (soil inoculation technique)

Mycoflora	Seedling vigour index						
	IPFD 10-12	Paras	KPMR 400	Indira matar	Shubhra	Ambika	Local variety
A.flavus	330.00	1216.00	1461.00	562.00	1045.00	1308.00	464.00
A.niger	232.00	684.00	492.00	654.00	785.00	684.00	117.00
Alternaria sp.	136.00	314.00	363.00	520.00	185.00	445.00	190.00
Chaetomium sp.	143.00	226.00	696.00	285.00	160.00	332.00	264.00
Trichoderma sp.	2668.00	3420.00	4820.00	4161.00	5132.00	4500.00	1864.00
Penicillium sp.	763.00	1300.00	2986.30	2120.00	1974.00	2325.00	580.00
Curvularia sp.	98.00	675.00	1165.00	208.00	172.00	297.00	126.00
Rhizopus sp.	101.00	218.00	680.00	91.00	52.50	190.00	124.00
Fusarium sp.	102.00	175.00	94.00	183.00	115.00	18.00	108.00
Control	965.40	1318.90	3079.10	2476.00	2031.20	2451.00	750.10

Table 2(b). Effect of seed borne mycoflora on seedling vigour of pea varieties (soil inoculation technique) (% increase or decrease over control)

Mycoflora	% increase (+) or decrease (-) over control							Mean increase/ decrease over control
	IPFD 10-12	Paras	KPMR 400	Indira matar	Shubhra	Ambika	Local variety	
A.flavus	-65.81	-7.80	-52.55	-77.30	-48.55	-46.63	-38.14	-48.11
A.niger	-75.96	-48.13	-84.02	-73.58	-61.35	-72.09	-84.40	-71.36
Alternaria sp.	-85.91	-76.19	-88.21	-78.99	-90.89	-81.84	-74.67	-82.38
Chaetomium sp.	-85.18	-82.86	-77.39	-88.48	-92.12	-86.45	-64.80	-82.46
Trichoderma sp.	176.36	159.30	56.53	68.05	152.65	83.59	148.5	120.71
Penicillium sp.	-20.96	-1.44	-3.10	-14.37	-2.79	-5.14	-22.67	-10.06
Curvularia sp.	-89.84	-48.82	-62.16	-91.59	-91.53	-87.88	-83.20	-79.28
Rhizopus sp.	-89.53	-83.47	-77.91	-96.32	-97.41	-92.24	-83.46	-88.62
Fusarium sp.	-89.43	-86.73	-96.94	-92.60	-94.33	-99.26	-85.60	-92.12

Table 3(a). Effect of seed priming on seedling vigour of pea varieties

Mycoflora	Seedling vigour index						
	IPFD 10-12	Paras	KPMR 400	Indira matar	Shubhra	Ambika	Local variety
Blitox-50 (COC	1711.20	2462.00	3883.50	5307.00	3653.00	4425.00	1161.80
SAAF(carbendazim +Mancozeb)	3097.00	1417.50	4511.00	4730.00	4713.00	4871.00	1199.60
Avtar (Hexaconazole+ Zineb)	1190.00	2311.00	4094.00	4202.00	3614.00	3234.00	1048.00
Bavistein (Carbendazim)	2451.00	1573.60	4413.00	3296.70	4295.00	4191.00	1966.00
Octave (Propineb)	1018.20	2390.00	4108.00	2891.20	4321.00	4857.00	1216.00
Trichoderma+ Vermicompost	2723.00	3870.00	4827.00	5608.00	4011.00	4564.00	1962.00
Control	951.30	1321.60	3063.60	2040.00	2401.60	2407.00	723.80

Table 3(b). Effect of seed priming on seedling vigour of pea varieties (percentage increase over control)

Treatments	% increase over control							Mean increase over control
	IPFD 10-12	Paras	KPMR 400	Indira matar	Shubhra	Ambika	Local variety	
Blitox-50(COC)	79.88	86.28	26.76	160.14	52.10	83.83	60.51	78.50
SAAF(carbendazim +Mancozeb)	225.55	7.25	47.24	131.86	49.04	102.36	65.73	89.86
Avtar(Hexaconazol +Zineb)	25.09	74.86	33.63	105.98	50.48	34.35	44.79	52.74
Bavistein (Carbendazim)	157.64	19.06	44.04	61.60	78.83	74.11	171.62	86.70
Octave (Propineb)	7.03	80.84	34.09	41.72	79.92	101.78	68.00	59.05
Trichoderma+ Vermicompost	186.23	192.82	57.55	174.90	67.01	89.61	171.06	134.1

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