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PREVALENCE OF SEED BORNE MYCOFLORA IN COMMERCIALLY CULTIVATED WHEAT VARIETIES AND MANAGEMENT OF BLACK POINT DISEASE CAUSED BY *Bipolaris sorokiniana*

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ABSTRACT

In the study, two sets of seed samples *viz.,* untreated and surface sterilized of different wheat seed samples were examined for the associated seed borne mycoflora by employing the Standard Purity Work Board and Standard Blotter Paper Method. The native best performing isolates of *Trichoderma* for their viability on different formulations and effect of suitable formulation on black point management studies were also studied. Dry Seed Examination (a non-destructive) method of detection, variety Raj 4037 was rated as resistant to seed-born mycoflora with 93.20 % healthy seed, 00.70 % deformed seed, 03.30 % wrinkled seed, 02.00% discoloured seed and 00.80 % fruiting body seed and variety WH 711 was rated as susceptible. Maximum 15 numbers of mycoflora *viz., Bipolaris sorokiniana, Alternaria alternata, Alternaria triticina, Curvularia lunata, Curvularia pallescens, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides, Epicoccum purpurascens, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.* were found associated by Standard Blotter Paper Method with the variety HD 2733 under untreated condition. Whereas Raj 4037 yielded minimum 8 numbers of pathogenic mycoflora. Talc powder (St.) formulation was found best for storage over long period of time followed by Talc powder (Us.) formulation. It recorded $21.11^{10}g^{-1}$ at IAP, $14.77^{10}g^{-1}$ at 30 DAP, $76.56^{9}g^{-1}$ at 60 DAP, $58.98^{8}g^{-1}$ at 90 DAP and $65.84^{6}g^{-1}$ CFU at 120 DAP whereas Talc powder (Us.) formulation recorded $17.78^{10}g^{-1}$ at IAP, $13.25^{10}g^{-1}$ at 30 DAP, $68.20^{9}g^{-1}$ at 60 DAP, $48.73^{8}g^{-1}$ at 90 DAP and $56.37^{6}g^{-1}$ CFU at 120 days after preparation (DAP) and these two formulation were rated as best formulation out of eight type of *T. harzianum* formulation. Out of best five formulation (on the basis of shelf life) tested for black point management, Talc Powder (St.) formulation was rated best, recorded significantly superior over the others with 78.00 % seed germination, 83.10 % shoot length, 11.63 cm spike length, 24.60 % back point incidence, 42.18 qtls/ha yield and 44.50 g test weight followed by Vermiculite formulation on the susceptible variety HD 2733.

KEYWORDS: Wheat, Black point disease, *Bipolaris sorokiniana* and *Trichoderma harzianum*

Wheat (*Triticum aestivum* L. em Thell.) is the most important cereal crop for the majority of countries. It is the most important staple food of about 36% of the world population. Worldwide, wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). According to geographical distribution of wheat cultivation, India is broadly divided into 5 wheat zones based on agro climatic condition:- North-Western Plains Zone, North Eastern Plain Zone, Central Zone, Peninsular Zone, Northern Hill Zone. At present with the change of cropping system, cropping intensity, crop management and varietal spread, *Bipolaris* is resulting in serious losses to wheat crop in the country as seed borne pathogen. In this context, the important concern is to manage the pathogens, which are major limiting factors in increasing the wheat productivity as well as production. At the same time, it is also necessary to know the dynamic of pathogenic seed borne mycoflora for holistic approach of management. Out of several seed borne wheat pathogens, *Bipolaris sorokiniana* is very much destructive and causing black point in wheat. Black Point in wheat (*Triticum aestivum* L) in an important seed-borne disease in all wheat growing regions of the world including India. The disease is mainly caused by *Bipolaris sorokiniana* (Sacc.) Shoeam. (Syn. *Helminthosporium sativum* teleomorph (*Cochliobolus sativus*) and *Alternaria* spp. For controlling black point disease, several approaches have been practiced, such as use of resistant variety, cultural control, chemical control, biological control and use of plant extract etc. Cultivation of resistant variety is the most acceptable method for controlling this disease. But none of the wheat varieties in the country is found resistant against this disease (Hossain and Azad, 1992). Vasetskaya, *et. al.,* 2001 reported that *Helminthosporium* is also producing carcinogenic toxins in wheat and barley grains. It has been reported that high degree of protection in many crops against a range of pathogens through seed treatment with spores of *Trichoderma harzianum* was achieved (Mukhopadhyay *et. al.,* 1992). Therefore, in the present study, the experiment were conducted to work out the seed

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borne mycoflora and to validate the technique of seed mycoflora detection in wheat along with the management of black point disease through biological control agent.

MATERIALS AND METHODS

The study was conducted to work out the distribution of mycoflora associated with the seed of ten commercially cultivated variety of wheat and management of Black Point disease of wheat. Seed samples of commercially cultivated variety of wheat were collected from the Directorate of Seed Research, Mau and were numbered, weighted and kept in paper envelop under low temperature condition in desiccators to avoid further contaminations and moisture. Seed of commercially cultivated wheat varieties *viz.,* PBW 343, PBW 550, DBW 17, UP 262, UP 2338, WH 711, HD 2733, Raj 3765, Raj 4037 and Raj 6516 were subjected for two detection techniques of seed-borne pathogenic mycoflora. The seed associated mycoflora were isolated from the infected seeds by using incubated Standard Blotter Paper Method and maintained on potato dextrose agar media. The fungal isolates were identified after growth on Czapak Dox Agar and Potato Dextrose Agar by using identification keys developed by Baijal and Mehrotra, 1980; Bissett, H. L. 1991; Bissett, J. 1991; Domsch, *et. al.,* 1993; Hammil, 1970 and Samuels, 1996. The pathogenicity test of *B. sorokiniana* was subjected on variety Sonalika and Koch's postulates were proved (Singh *et. al.,* 2013)

Standard Purity Work Board (Dry Seed Examination)

All the samples were subjected to the visual inspection by naked eye, under stereoscopic binocular microscope added with cool-light condition and by modified purity work board. Seeds with black point disease can be easily differentiated by the pronounced appearance of brown to dark brown or blackish discoloured areas. Symptoms on the seeds, having elliptical to oblong lesions with lighter in center represent the presence of *Bipolaris sorokiniana*. *Curvularia lunata* resulted brown discoloration of seed coats. *Alternaria alternata* was identified by the presence of dark brown and long conidial chain on incubated seed surface.

Standard Blotter Paper Method (ISTA 2015)

It is an incubation method in which untreated and treated (surface sterilization @ 0.01 % $HgCl_2$) seeds are placed on well water soaked filter papers, and incubated for 7 days at 24 ± 1 ^{0}C under 12 h alternating cycles of light and darkness. After incubation, fungi developed on each seed were examined under different magnification of a stereoscopic microscope and identified. The identification of the fungi is based on the way they grow on seeds "habit characters", and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope.

Management of Black Point in Wheat

Bio-control agent *T. harzianum* (Bissett, H. L. 1991 and Bissett, J. 1991) was isolated from the rhizospheric soil of wheat by following the standard protocol of serial dilution and maintained in the Directorate of Seed Research Laboratory, Mau. Sum total of fifteen different *T. harzianum* isolates were isolated and deployed for the dual culture studies (Fokkema, 1978) against the black point pathogen. Out of these isolates, one potential isolate of *T. harzianum* having maximum antagonistic potential was used for the management study. It was maintained on potato dextrose agar (PDA) slants. The seed dressing formulations were prepared by mixing their ingredients. Before adding carboxy methyl cellulose, the fine mixture (200 mesh) of substrates was dried for 12 h at 60°C in a hot air oven. A fine powder of the substrates was made in a grinder. The mixture of substrates was inoculated with the inoculum of *Trichoderma* species grown on sorghum (*Sorghum vulgare* Pers.) grains (Dubey *et. al.,* 2009).

Shelf Life Studies of Different Formulation

The viability of different formulation *viz.,* Talc power (St), Talc power (Us), Peat power, Multani soil, Lignite, Vermiculite, Wheat bran and Rice bran had been studied. The CFU immediate after formulation, 30 days after formulation, 60 days after formulation, 90 days after formulation and 120 days after formulation under laboratory condition was worked out by enumeration using serial dilution technique. The water activity and percent moisture content were also recorded during the shelf life studies. The water activity was measured using water activity meter (HW3 model of Rotronic make) and the moisture content was recorded using Sartorius moisture analyzer for the accuracy of the study.

Effect of Suitable Formulation on Black Point Incidence

The pot experiments were conducted in pollyhouse of Directorate of Seed Research, Mau in completely randomized design (CRD) to evaluate the performance of different seed dressing formulations of *T. harzianum.* Eight different formulations *viz.,* Talc power (St), Talc power

(Us), Peat power, Multani soil, Lignite, Vermiculite, Wheat bran and Rice bran developed from the potential isolate of *Trichoderma* were evaluated. The seeds of most susceptible variety HD 2733 were coated with the different formulations of *T. harzianum* and sown in the sterilized pots. Recommended agronomic practices of wheat were follows to raise the good crop. *B. sorokiniana*, isolated from wheat seed was cultured on potato dextrose agar at 26 °C. Cultures used for inoculum preparation were derived from a single conidium. Cultures were incubated under alternating cycles of 12 h of light and 12 h of darkness for at least 10 days. Conidia were harvested into phosphate buffer with a sterile soft-hair brush, and suspensions were filtered through four layers of sterile cheese cloth to remove hyphal fragments. Spore concentrations were determined with a hemacytometer and adjusted to 5×10^5 spores per ml for plant inoculations (Zhang and Yuen, 1999). The spore suspension was sprayed at 3 to 4 leaf stage of wheat crop by a hand-operated atomizer. The second and third inoculation was made again in the same manner each after fifteen days. Each time the pot was irrigated just after inoculation to create favourable humidity for disease development. The crop was regularly observed after germination up to harvesting. The observation on different parameters were recorded. The black point incidence was recorded (Singh *et al.,* 2013).

RESULTS

Detection of Mycoflora by Standard Purity Work Board

The commercially cultivated wheat seed samples of all the varieties were analyzed on Standard Purity Work Board under dry conditions and healthy and diseased seeds were sorted out. Diseased seeds were deformed, shriveled, deshaped and discoloured. Seeds with black point disease can be easily differentiated by the pronounced appearance of brown to dark brown or blackish discoloured areas. Symptoms on the seeds, having elliptical to oblong lesions with lighter in centre represent the presence of *Bipolaris sorokiniana*. *Curvularia spp.* resulted brown discoloration of seed coats. *Alternaria alternata* was identified by the presence of dark brown and long conidial chain on incubated seed surface. The associations of above mentioned mycoflora were finally confirmed after plating of seeds (stored sample) by using standard blotter paper method. Maximum fruiting body seed was recorded 19.66 % with the variety PBW 343 followed by 03.90% with variety WH 711 and 2.40 % with Raj 3765 (Table 1.). Statistically significant minimum fruiting body seed was recorded 00.60 % with the three variety PBW 550, UP 2338 and Raj 6516 followed by 00.80 % (Raj 4037). The minimum discoloured seed was recorded 00.60 % with the two variety HD 2733 and UP 262 followed by 00.90 % (PBW 550) whereas maximum discoloured seed was observed 07.30 % with the variety WH 711 followed by 3.10 % with the variety UP 262 and 02.30 % with the variety Raj 3765. The discoloured seed percentages were statistically non-significant with each of the variety. The maximum wrinkled seed was recorded 20.70 % with the variety WH 711 followed by 10.20 % (UP -2338) and 05.20 % (Raj 3765). Whereas the minimum wrinkled seed was recorded 02.70 % with the variety PBW 343 followed by 03.10 % (DBW 17) and 03.20 % (Raj 6517). The maximum and minimum differences of wrinkled seed in the studied varieties was statistically significant to each other. In case of deformed seed, 06.20 % maximum deformed was observed with the variety WH 711 followed by 05.60 % (HD 2733) and 03.70 % (DBW 17) and statistically non-significant with the minimum deformed seed of 00.50 % with the variety PBW 343 followed by 00.70 % (Raj 4037) and 01.00 % (Raj 3765, Raj 6516 and UP 2338).

The maximum healthy seed of 93.20 % was recorded with the variety Raj 4037 followed by 93.10 % (Raj 6516) and 92.60 % (PBW 550) and statistically significant with the minimum 61.90 % healthy seed with the variety WH 711 followed by HD 2733 and UP 2338.

Overall under the dry seed examination (a non-destructive) method of detection, variety Raj 4037 was rated as resistant to seed-born mycoflora with 93.20 % healthy seed, 00.70 % deformed seed, 03.30 % wrinkled seed, 02.00% discoloured seed and 00.80 % fruiting body seed and variety WH 711 was rated as susceptible with 61.90 % healthy seed, 06.20 % deformed seed, 20.70 % wrinkled seed, 07.30 % discoloured seed and 03.90 % fruiting body seed.

Table 1: Dry seed examination of fruiting body seed, discoloured seed, wrinkled seed, deformed seed, and healthy seed of ten commercially cultivated wheat varieties through standard purity work board

Sl. No.	Variety	SE	FBS	DS	WS	DFS	HS
1	PBW 343	1000	19.66	01.60	02.70	00.50	89.30
2	PBW 550	1000	00.60	00.90	04.60	01.30	92.60
3	DBW 17	1000	00.90	02.20	03.10	03.70	90.10
4	UP 262	1000	02.30	03.10	04.30	01.10	89.30
5	UP 2338	1000	00.60	00.60	10.20	01.00	87.60
6	WH 711	1000	03.90	07.30	20.70	06.20	61.90
7	HD 2733	1000	01.70	00.60	04.90	05.60	87.20
8	Raj 3765	1000	02.40	02.30	05.20	01.00	89.10
9	Raj 4037	1000	00.80	02.00	03.30	00.70	93.20
10	Raj 6516	1000	00.60	02.10	03.20	01.00	93.10
Mean			06.53	07.26	20.73	07.36	958.10
SEm±			2.37	4.527	6.737	3.214	7.970
$CD_{0.01}$			6.74	12.83	19.11	9.11	22.63

SE = Seeds examined; FBS = Fruiting body seed; DS = Discoloured seed; WS = Wrinkled seed; DFS = Deformed seed; HS = Healthy seed

Detection of Mycoflora by Standard Blotter Paper Method

Seed samples of commercially cultivated wheat varieties were analyzed for the associated mycoflora (Table 2.). Among the samples of different varieties tested, maximum, fifteen (15) mycoflora were recorded under untreated condition in variety HD 2733 seeds with 88.00 % germination and 17.00 % diseases seed followed by 12 mycoflora in variety PBW 343 with 93.60 % germination and 12.60 % diseased seeds. *Bipolaris sorokiniana, Alternaria alternata, Alternaria triticina, Curvularia lunata, Curvularia pallescens, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides, Epicoccum purpurascens, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.* were the mycoflora found associated with the untreated seeds of variety HD 2733. Whereas under treated seed condition, HD 2733 yielded maximum 11 numbers of mycoflora with 90.60 % germination and 12.00 % diseased seed followed by PBW 343 with 96.30 % germination and 11.60 % diseased seed. *Bipolaris sorokiniana, Alternaria alternata, Alternaria triticina, Curvularia lunata, Curvularia pallescens, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides, Aspergillus Niger* and *Rhizopus spp.* were the mycoflora with treated seeds of the variety HD 2733. Minimum eight mycoflora *viz., Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Trichoderma hamatum, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.* were isolated from Raj 4037 under untreated seed with 92.60 % germination and 02.00 % diseased seed followed by DBW 17 with 93.60 % germination and 03.00 % diseased seed. Under treated condition minimum 6 pathogenic mycoflora *viz., Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Trichoderma hamatum, Aspergillus flavus,* and *Rhizopus spp.* were isolated in variety Raj 4037 with 94.60 % germination and 1.60 % disease seed followed by four varieties DBW 17, UP 262, UP 2338 and Raj 3765. Statistically significant and maximum 94.60 % germination with the variety UP 262 and minimum 88.00 % germination with HD 2733 was recorded under untreated condition whereas maximum 96.60 % germination of variety PBW 550 and minimum 90.06 % germination of variety HD 2733 was recorded under treated conditions of the seeds. Under untreated condition, maximum 17.00 %, statistically significant diseased seed was recorded with the variety HD 2733 as compared to the minimum 02.00 % disease seed of Raj 4037. These were at par with the variety Raj 6516 (13.00%) and variety DBW 17 (03.00 %), respectively.

Overall, variety HD 2733 yielded maximum 15 numbers of mycoflora (88.00 % germination and 17.00 % diseased seed) under untreated condition and 11 numbers of mycoflora (90.60 % germination and 12.00 % diseased seed) under treated condition and rated as susceptible variety. Whereas Raj 4037 yielded minimum 8 numbers of mycoflora (92.60 % germination and 02.00 % diseased seed) under untreated condition and 6 numbers of mycoflora (94.60 % germination and 01.60 % diseased seed) under treated condition and rated as resistant variety. *Fusarium oxysporum* was found associated in all the

varieties in both the condition of seed, either in untreated condition or in treated condition except variety UP 262.

Table 2: Seeds associated mycoflora of commercially cultivated wheat variety by Standard Blotter Paper Method before and after surface sterilization

Sl. No.	Variety	Type of seed	Seeds examined	Germination %	Seed/Seedling %		Mycoflora isolated
					Normal	Diseased	
1	PBW 343	Un-treated	300	93.60	87.40	12.60	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	96.30	88.40	11.60	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Nigrospora oryzae, Aspergillus flavus,* and *Rhizopus spp.*
2	PBW 550	Un-treated	300	94.30	90.80	09.20	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	96.60	93.80	06.20	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Aspergillus flavus,* and *Rhizopus spp.*
3	DBW 17	Un-treated	300	93.60	97.00	03.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Trichoderma hamatum, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	96.30	97.70	02.30	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger,* and *Rhizopus spp.*
4	UP 262	Un-treated	300	94.60	92.50	07.50	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Macrophomina phaseolina, Trichoderma hamatum, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	96.30	96.00	04.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Macrophomina phaseolina, Trichoderma hamatum, Aspergillus flavus,* and *Rhizopus spp.*
5	UP 2338	Un-treated	300	90.30	89.00	11.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	92.30	93.00	07.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Trichoderma hamatum, Aspergillus niger,* and *Rhizopus spp.*
6	WH 711	Un-treated	300	91.60	92.00	08.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	95.30	93.40	06.60	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Trichoderma hamatum, Nigrospora oryzae, Aspergillus niger,* and *Rhizopus spp.*
7	HD 2733	Un-treated	300	88.00	83.00	17.00	*Bipolaris sorokiniana, Alternaria alternata, Alternaria triticina, Curvularia lunata, Curvularia pallescens, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides,*

							Epicoccum purpurascens, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp. and *Rhizopus spp.*
		Treated	300	90.60	86.00	12.00	*Bipolaris sorokiniana, Alternaria alternata, Alternaria triticina, Curvularia lunata, Curvularia pallescens, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides, Aspergillus niger* and *Rhizopus spp.*
8	Raj 3765	Un-treated	300	93.00	93.00	06.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	95.30	94.00	04.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Trichoderma hamatum, Aspergillus niger,* and *Rhizopus spp.*
9	Raj 4037	Un-treated	300	92.60	98.00	02.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Trichoderma hamatum, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	94.60	98.40	01.6.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Trichoderma hamatum, Aspergillus flavus,* and *Rhizopus spp.*
10	Raj 6516	Un-treated	300	93.30	87.00	13.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Cladosporium cladosporioides, Nigrospora oryzae, Aspergillus flavus, Aspergillus niger, Penicillium spp.* and *Rhizopus spp.*
		Treated	300	95.60	92.00	08.00	*Bipolaris sorokiniana, Alternaria alternata, Curvularia lunata, Fusarium oxysporum, Macrophomina phaseolina, Trichoderma hamatum, Aspergillus flavus,* and *Rhizopus spp.*
Mean				93.78		7.81	
SEm±				1.42		2.32	
$CD_{0.01}$				4.03		6.58	

Shelf life studies of different formulation

Viability of *Trichoderma harzianum* spores under different formulation *viz.,* Talc power (St), Talc power (Us), Peat power, Multani soil, Lignite, Vermiculite, Wheat bran and Rice bran had been studied. The CFU was worked out immediate after formulation, 30 days after formulation, 60 days after formulation, 90 days after formulation and 120 days after formulation under ambient condition (Table 3.). Loss of viability of *T. harzianum* spores was recorded with the advancement of storage age of formulation. Immediate after formulation, Rice Bran formulation was recorded the highest CFU $77.88^{10}g^{-1}$ followed by $46.15^{10}g^{-1}$ of Multani Soil as compare to the minimum $14.16^{10}g^{-1}$ followed by Talc powder (Us.) as $17.70^{10}g^{-1}$. After 30 days advance of age Vermiculite formulation was recorded highest $19.51^{10}g^{-1}$ followed by Rice Bran formulation ($18.11^{10}g^{-1}$) as compare to the minimum of $0.9.13^{10}g^{-1}$ of Wheat Bran formulation followed by $09.91^{10}g^{-1}$ of Multani Soil formulation. At the age of 60 days after formulation, maximum CFU of $76.56^{9}g^{-1}$ was recorded in Talc powder (St.) formulation followed by $68.20^{9}g^{-1}$ of Talc powder (Us.) formulation and statistically significant over the minimum CFU of $23.66^{9}g^{-1}$ of Multani Soil followed by $30.50^{9}g^{-1}$ CFU of Wheat Bran formulation. By attaining the 90 days after formulation, Talc Powder (St.) recorded highest $58.90^{8}g^{-1}$ CFU followed by $48.73^{8}g^{-1}$ CFU of Talc Powder (Us.). Whereas minimum CFU was recorded in Multani Soil ($16.62^{8}g^{-1}$) followed by $19.61^{8}g^{-1}$ of Wheat Bran formulation. Maximum and minimum difference of CFU was statistically significant to each other. One hundred twenty days after formulation, maximum $65.84^{6}g^{-1}$ CFU was observed in Talc Powder (St.) formulation followed by $56.37^{6}g^{-1}$ CFU of Talc Powder (Us.0 formulation as compare to minimum of 17.66 $^{8}g^{-1}$ CFU of Multani Soil formulation followed by $19.61^{6}g^{-1}$ CFU of Wheat Bran.

Viability of *T. harzianum* spores was found more alive in Talc powder (St.) formulation over long period of

time followed by Talc powder (Us.) formulation. At the early stage of formulation, both these formulation was recorded low CFU. Talc Powder (St.) was recorded $21.11^{10}g^{-1}$ at IAP, $14.77^{10}g^{-1}$ at 30 DAP, $76.56^{9}g^{-1}$ at 60 DAP, $58.98^{8}g^{-1}$ at 90 DAP and $65.84\ ^{6}g^{-1}$ CFU at 120 DAP whereas Talc powder (Us.) formulation recorded $17.78^{10}g^{-1}$ at IAP, $13.25^{10}g^{-1}$ at 30 DAP, $68.20^{9}g^{-1}$ at 60 DAP, $48.73^{8}g^{-1}$ at 90 DAP and $56.37^{6}g^{-1}$ CFU at 120 days after preparation (DAP) and these two formulation were rated as best formulation out of eight type of *T. harzianum* formulation.

Table 3: Viability of different formulation of *T. harzianum* under laboratory condition

Sl. No.	Bio-agent formulation	CFU (10^{10} g^{-1} formulation) IAP	CFU (10^{10} g^{-1} formulation) 30 DAP	CFU (10^{9} g^{-1} formulation) 60 DAP	CFU (10^{8} g^{-1} formulation) 90 DAP	CFU (10^{6} g^{-1} formulation) 120 DAP
1	T_1 (Talc powder St)	21.11	14.75	76.56	58.98	65.84
2	T_2 (Talc powder Us)	17.78	13.25	68.20	48.73	56.37
3	T_3 (Peat power)	25.72	16.05	52.45	35.73	34.63
4	T_4 (Multani soil)	46.15	09.91	23.66	16.62	17.66
5	T_5 (Lignite)	24.22	16.89	60.43	42.47	49.51
6	T_6 (Vermiculite)	37.65	19.51	40.47	24.78	24.78
7	T_7 (Wheat bran)	14.16	09.13	30.50	19.61	19.61
8	T_8 (Rice bran)	77.88	18.11	38.82	24.40	24.40
Mean		19.76	13.19	49.30	34.71	36.603
SEm±		00.77	00.72	00.86	00.79	01.32
$CD_{0.01}$		02.24	02.10	02.51	02.30	03.85

IAP= Immediate After Preparation; DAP= Days After Preparation

Effect of suitable formulation on black point incidence

Effect of five formulations of *T. harzianum* on seed germination, shoot and spike length, yield, test weight and black point incidence was studies on variety HD 2733 under field conditions (Table 4). Maximum germination of 78.00% was recorded after seed coating of Tale powder (St.) followed by 69.00% with Vermiculite formulation and statistically significant with the minimum of 57.33% germination with Talc Powder (Us.) followed by Peat Powder formulation. In respect to shoot and spike length, maximum 83.10 cm and 11.63 cm were recorded with Talc Powder (St.) formulation followed by 80.96 cm and 09.53 cm with Vermiculite formulation, respectively and statistically significant with minimum 58.06 cm shoot length and 07.80 cm spike length of Talc Powder (Us.) formulation. Minimum black point incidence of 24.00% was observed with coating of Talc Powder (St.) formulation followed by 34.33 % black point incidence of Vermiculite formulation coating seed. These were statistically significant over the maximum black point incidence of 78.33 % with control. Talc Powder (St.) coated seed recorded significantly lower incidence of black point as compare to four other formulations. Significant increase of yield was recorded through seed coating of *T. harzianum* formulation over the control of 23.10 qtls/ha. Maximum 42.18 qtls/ha yield was recorded in Tale Powder (St.) coated seeds followed by 39.60 qtls/ha by Vermiculite formulation coating. Test weight of seeds was also significantly increased after coating of seed with Tale Powder (St.) formulation, recorded maximum 44.55 g as compare to the minimum 19.96 g over control.

Tale Powder (St.) formulation was rated best amongst the other formulations, recorded significantly superior of 78.00 % germination, 83.10 % shoot length, 11.63 cm spike length, 24.60 % back point incidence, 42.18 qtls/ha yield and 44.50 g test weight followed by Vermiculite formulation with 69.00 % germination, 80.96 % shoot length, 09.53 cm spike length, 34.33 % back point incidence, 39.60 qtls/ha yield and 39.66 g test weight.

Table 4: Effect of suitable formulation of *T. harzianum* on seed germination shoot and spike length, yield, test weight and black point incidence on variety HD-2733

Sl. No.	Bio-agent formulation	Seed Germination%	Shoot length (cm)	Spike length (cm)	Black point incidence %	Yield (Qtls/ha)	1000 grain weight (g)
1	T_1 (Talc power St)	78.00	83.10	11.63	24.60	42.18	44.55
2	T_2 (Talc power Us)	57.33	58.06	07.80	55.66	29.27	26.50
3	T_3 (Lignite)	75.00	69.33	08.56	45.66	35.47	32.79
4	T_4 (Peat power)	62.66	68.93	07.23	65.33	28.01	25.83
5	T_5 (Vermiculite)	69.00	80.96	09.53	34.33	39.60	39.66
6	*T_6 Control	50.00	41.06	05.90	78.33	23.10	19.96
Mean		65.33	65.41	8.44	50.66	32.94	31.54
SEm±		03.64	02.04	00.50	03.02	01.23	01.53
$CD_{0.01}$		11.10	06.24	01.52	09.21	03.75	04.66

*Control = Untreated diseased seed

DISCUSSION

In the study, two sets of seed samples (untreated and surface sterilized) of different wheat seed samples were examined for the associated seed borne mycoflora by employing the Standard Purity Work Board, Standard Blotter Paper Method. Shelf life of different formulation and effect of suitable formulation on black point incidence was also worked out.

Overall under the dry seed examination (a non-destructive) method of detection, variety Raj 4037 was rated as resistant to seed-born mycoflora with 93.20 % healthy seed, 00.70 % deformed seed, 03.30 % wrinkled seed, 02.00% discoloured seed and 00.80 % fruiting body seed. Variety WH 711 was rated as susceptible in respect to seed abnormalities with 61.90 % healthy seed, 06.20 % deformed seed, 20.70 % wrinkled seed, 07.30 % discoloured seed and 03.90 % fruiting body seed. Detection of Mycoflora by Standard Blotter Paper Method, variety HD 2733 yielded maximum 15 numbers of pathogenic mycoflora (88.00 % germination and 17.00 % diseased seed) under untreated condition and 11 numbers of pathogenic mycoflora (90.60 % germination and 12.00 % diseased seed) under treated condition and rated as susceptible variety .Whereas Raj 4037 yielded minimum 8 numbers of pathogenic mycoflora (92.60 % germination and 02.00 % diseased seed) under untreated condition and 6 numbers of pathogenic mycoflora (94.60 % germination and 01.60 % diseased seed) under treated condition and rated as resistant variety. *Fusarium oxysporum* was found associated in all the varieties in both the condition of seed, either in untreated condition or in treated condition except variety UP 262. The results obtained are in close conformity with those of Limonard (1968) who reported that the use of 0.1 % $HgCl_2$ solution effectively reduced the microbial contamination. Use of 0.1 % $HgCl_2$ solution helped in minimizing the incidence of superficial and fast growing as well as common seed-borne fungi like, *Fusarium spp., Aspergillus spp.* and *Curvularia lunata.* Similar findings were also reported by Zafar *et. al*., (2014), *et. al*., (2005) and Dawar and Gaffar (1991).

Shelf life of *T. harzianum* spores was found maximum in Talc powder (St.) formulation over long period of time followed by Talc powder (Us.) formulation. At the early stage of formulation, both these formulation was recorded low CFU. Talc Powder (St.) was recorded $21.11^{10}g^{-1}$ at IAP, $14.77^{10}g^{-1}$ at 30 DAP, $76.56^{9}g^{-1}$ at 60 DAP, $58.98^{8}g^{-1}$ at 90 DAP and 65.84 $^{6}g^{-1}$ CFU at 120 DAP whereas Talc powder (Us.) formulation recorded $17.78^{10}g^{-1}$ at IAP, $13.25^{10}g^{-1}$ at 30 DAP, $68.20^{9}g^{-1}$ at 60 DAP, $48.73^{8}g^{-1}$ at 90 DAP and $56.37^{6}g^{-1}$ CFU at 120 days after preparation (DAP) and these two formulation were rated as best formulation out of eight type of *T. harzianum* formulation. Tale Powder (St.) formulation was rated best form other formulations, recorded significantly superior of 78.00 % germination, 83.10 % shoot length, 11.63 cm spike length, 24.60 % back point incidence, 42.18 qtls/ha yield and 44.50 g test weight followed by Vermiculite formulation. Circumstantial evidence indicated that *S. atra was antagonistic to H. sativum* by the production of an toxic substances (Domsch *et. al.,* 1980). On PDA, *T. harzianum* inhibited *H. sativum* and then grew over it, which indicates that *T. harzianum* is the more aggressive saprophyte in culture, and also appeared to be antagonistic by its occupation of the substrate, production of an antibiotic (Dossantos and Dhingra, 1982). The antagonistic

effect of antagonists interferes with successive phases of the development of pathogen. Presence of metabolites within the culture filtrates have also been shown to affect plant pathogenic fungi (Dossantos and Dhingra, 1982). Bell *et. al.,* (1982) demonstrated that species of *Trichoderma* or their antibiotics, suppressed growth of six fungal plant pathogens. Similar results were reported by Luz *et. al.,* 1998), who suggested that colonization of infected wheat seeds may be controlled by secretion of antibiotics from the antagonistic microorganisms associated with seeds. It is possible that all the antifungal activity on seeds is due to antagonist's growth and antibiotic production. Some inhibition could result from toxic compounds accumulated in the culture medium showing biological activity against pathogens. Present of biocontrol agent at or near the pathogen's infection court, where they act by producing antifungal or antibiotic compounds, through hyper parasitism, or by competitively colonizing spermosphere and rhizosphere substrates (Taylor and Harman 1990). Seed treatment is an attractive delivery system of fungal bio-protectants (Wright *et. al.,* 2003).

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