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EVALUATION OF THE EFFECT OF DIFFERENT CARRIER MATERIALS ON VIABILITY AND RESISTANCE INDUCTION POTENTIAL OF *PENICILLIUM OXALICUM*

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A B S T R A C T

Preparation of inoculum of biocontrol species in the most suitable carrier material is imperative to get full benefit from its resistance induction potential. Present study focuses upon the evaluation of supportive behavior of carrier materials towards *Penicillium oxalicum* Currie & Thom in controlling Alternaria leaf spots of tomato caused by *Alternaria alternata* (Fr.) Keissl. Inoculum of resistance inducer species was prepared in five different carrier materials namely talc, bentonite, sand, saw dust and Bhagaas; and incubated at room temperature for 0, 10, 20…80 days. Then soil applications of all those treatments under the field conditions revealed that Bhagaas based inducer inoculum with no incubation period controlled fungal disease most efficiently. Furthermore, evaluation of agro-economic aspects of tomato plants also proved Bhagaas as the best carrier material by which high yield and maximum vigor index was achieved. Second most efficient carrier was saw dust followed by talc, sand and bentonite, respectively. Present study concludes that inoculum of *P. oxalicum* should be prepared using Bhagaas as cerrier material.

Keywords: Agro-economic characteristics, biocontrol, carrier material, disease incidence, Incubation, Resistance inducer.

INTRODUCTION

Alternaria alternata (Fr.) Keissl. is a pre as well as post-harvest fungal pathogen of tomato with significant annual losses (Bautista-Baňos *et al*., 2006). It attacks tomato leaves and reduces the photosynthetic area of tomato plants by developing concentric rings. These rings have yellow outer margin and coalesce to develop dead necrotic leaves (Rabea and Steurbaut, 2010). It is a devastating pathogen with a wide host range, and it may also survive in plant debris as well. It is a cause of serious annual losses of tomato crop, therefore it is very important to be controlled (Akhtar *et al.,* 1994, 2004).

Controlling plant pathogens by using microbial species inducing systemic resistance in plants is a preferred technique due to its effective control, low cost and environment friendly behavior (Narayanasamy, 2008). For this purpose *P. oxalicum* is a well-known disease biocontrol agent (Ade *et al*., 1997; Sabuquillo *et al*., 2005). It has been successfully used to control fungal and bacterial pathogens e.g. *Alternaria* species, *Phytophthora* species and *Ralstonia solanacearum* etc. on many plants including tomato and cucumber. It has been reported to provide more than 60% control of the tested pathogens. However, its field applications require its inoculum prepared in suitable carrier material (Rabea and Steurbaut, 2010). These carrier materials make their handling easier and provide them sufficient nutrition to retain their efficiency. Therefore, carrier medium used for the application of biocontrol agent largely affects the results expected from inducers (Espinel-Ingroff *et al*., 2004). Therefore, it is imperative to optimize carrier material conditions to get the best from this biocontrol agent. The present study was performed to evaluate the potential of different carrier materials to support resistance induction behavior of *P. oxalicum* in tomato plants against *A. alternata*.

MATERIALS AND METHODS

Procurement of materials: Seeds of two tomato varieties with differential antifungal resistance (Dinaar = resistant; Red Tara= susceptible) were procured from Agriculture Market, Multan Road, Lahore. Inoculum of *P. oxalicum* and *A. alternata* were also obtained from Fungal Biotechnology Research Lab, University of the

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Punjab, Lahore, Pakistan, for evaluating the efficacy of carrier materials. Five different types of carrier materials namely talc, bentonite, sand, saw dust, bhagaas were tested for their tendency to support resistance induction feature of *P. oxalicum*.

Experimental setup: Inoculum of *P. oxalicum* was prepared in all types of carrier materials (talc, bentonite, sand, saw dust, bhagaas) with the difference of consecutive ten days. For this purpose, 1 Kg of each carrier material was inoculated with *P. oxalicum* under aseptic conditions and incubated for one week at 25 °C. The prepared inocula were stored at room temperature in order to setup resistance induction efficacy test of fungus in different carrier materials. In this way, 9 *Penicillium* inocula of different age were prepared. Seeds of the two tomato varieties were sown in pots of 28 cm diameter. After one month, pots were divided into 102 treatments in which each variety had a share of 51 treatments; while a single treatment consisted of three pots in it. Then 51 treatments of single variety were further distributed into five groups with 10 treatments in each group; remaining one treatment as a positive control (PC) with pathogen inoculation only. Furthermore, 10 treatments of each group were treated with nine *Penicillium* inoculum of different age and one control treatment of carrier material only. Five grams of *P. oxalicum* inoculum and 5 mL of pathogen inoculum (3×10^3 spores mL^{-1}) were applied to each plant at one time according to experimental design.. After two weeks data were recorded regarding disease incidence and percentage control was calculated by following formula. Data were further statistically analyzed and carrier material with long term and efficient control was evaluated as the best carrier material for *P. oxalicum*.

$$\text{Percentage control} = \frac{\text{DI of PC} - \text{DI of carrier treatment}}{\text{DI of PC}} \times 100$$

DI= Disease Incidence

PC= Positive Control

Agro-economic traits: After recording data regarding disease incidence plants were transplanted in fields and watered when required. After that period data were recorded regarding Agro-economic traits in terms of plant height, stem diameter (at 5 cm from soil surface), number of leaves per plant, number of fruits per plant, number of branches, average weight of five fruits, chlorophyll contents and number of leaf hairs per unit leaf area.

Chlorophyll contents were measured in mg g^{-1} by adopting the method of Bojović and Stojanović (2005). Efficiencies of all carrier materials were calculated after measuring all the Agro-economic traits and the best carrier material was evaluated by taking the sum of all scores obtained by individual carrier according to grading table (Table 1).

Table 1. Efficiency score table of carrier materials.

Plant Height (cm)		Stem Diameter (cm)		No. of Branches Plant^{-1}		Chlorophyll contents (mg g^{-1})		No.of Hairs (9 mm^{-2})		No. of Fruits Plant^{-1}		Weight of five fruits (g)		Number of leaves	
Scale	Status	Scale	Status	Scale	Status	Scale	Status	Scale	Status	Scale	Status	Scale	Status	Scale	Status
51-60	1	0.9-1	1	15-18	1	0.3-0.4	1	3-6	1	31-50	1	41-85	1	71-90	1
61-70	2	1.1-1.2	2	19-22	2	0.5-0.6	2	7-10	2	51-70	2	86-130	2	91-110	2
71-80	3	1.3-1.4	3	23-26	3	0.7-0.8	3	11-14	3	71-90	3	131-175	3	111-130	3
81-90	4	1.5-1.6	4	27-30	4	0.9-1	4	15-19	4	91-110	4	176-220	4	131-150	4
91-100	5	1.7-1.8	5	31-34	5	1.1-1.2	5	20-24	5	111-130	5	221-265	5	151-170	5
101-110	6	1.9-2	6	35-38	6	1.3-1.4	6	25-29	6	131-150	6	266-310	6	171-190	6

Statistical Analysis: All the data was analyzed for its significance with Analysis of Variance (ANOVA) and LSD Test using DSAASTAT (Onofri, Italy).

RESULTS

Maximum disease control was shown by *P. oxalicum* when its bhagaas and saw dust formulations were applied after zero day incubation period (Table 2). Resistance induction activity was more persistent in Bhagaas than saw dust based inoculums of *P. oxalicum* because after 80 days of incubation Bhagaas supported approximately 90% disease controlling activity of inducer; while Saw Dust could facilitate 80% disease controlling activity of *P. oxalicum*. Sand proved as a best carrier only for short incubation periods and its efficacy was sharply decreased from 0-50 days of incubation. For more incubation days no further decrease in percentage disease control was observed (Table 2).

Table 2. Percentage diseases control by *Penicillium oxalicum* in combination with different carrier materials and different incubation periods.

Inoculum Incubation (days)	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	70.7	70.9	80.6	76.9	97.2	90.5	100	100	100	100
10	68.4	62.5	71.3	71.6	70.1	67.9	100	99.4	100	100
20	65.8	63.2	68.9	62.4	40.3	43.2	97.4	95.1	99.8	100
30	57.1	57.3	59.6	56.6	12.4	16.7	90.3	92.7	100	97.6
40	56.3	54.9	54.7	51.7	09.5	04.5	88.2	90.5	99.4	96.8
50	56.3	53.3	51.2	40.2	03.1	0.00	87.6	86.9	98.1	96.8
60	51.2	50.5	43.7	32.1	0.00	0.00	83.1	86.2	96.7	92.3
70	50.7	47.8	36.9	30.1	0.00	0.00	82.4	84.4	95.9	89.7
80	49.7	43.6	23.1	26.4	0.00	0.00	80.1	81.8	95.4	87.2
Carrier Control	3.1	2.9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data were analyzed statistically and LSD value was 1.28.
DS on PC of resistant (Dinaar) was 18.09, while IC and NC showed zero DI
DS on PC, IC and NC of susceptible (Red Tara) was 6.0, whereas IC and NC exhibited zero DI

Bentonite and talc did not prove better carriers than sand at short incubations and provided lesser disease control also. However, after 50 days of incubation, almost 50% and 37% control was recorded by Talc and Bentonite, respectively. Talc and Bentonite even controlled disease incidence after 80 days of incubation. It was also notable that Talc was a relatively better carrier than Bentonite and also provided diseases protection up to some extent even without *P. oxalicum* inoculum. It revealed that Talc also had itself some tendency to trigger plant defense system against pathogens; but, Talc, *P. oxalicum* combination still failed to show better results than all other carrier materials at zero day of incubation. Data presented in Table 2 also shows the inert behavior of bentonite, sand, saw dust and Bhagaas with tomato plants regarding induction of resistance against fungal diseases.

All the other parameters studied viz. height of tomato plants (Table 3), stem diameter (Table 4), number of leaves/plant (Table 5), number of fruits/plant (Table 6), number of braches/plant (Table 7), weight of 5 fruits (Table 8), chlorophyll contents (Table 9) and number of leaf hairs (Table 10) showed similar pattern as described in Table 2.

Table 3. Height of tomato plants (cm) subjected to different treatments of carrier materials with *Penicillium oxalicum* and incubation periods.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	74.6	69.5	84.4	79.8	103.7	97.6	108.4	108.6	109	108.7
10	71.5	68.4	79.4	78.7	94.9	96.4	101.4	106.7	109.1	107.9
20	70.3	68.1	77.6	76.8	87.1	91.3	97.6	102.7	107.3	105.7
30	66.6	65.3	71.4	74.1	83.9	82.7	97.6	98.5	103.5	104.1
40	64.7	63.9	72.4	68.1	76.5	71.3	95.4	97.1	104.1	101.6
50	65.0	60.7	61.7	64.7	72.8	68.8	91.7	92.7	102.1	95.6
60	62.1	59.4	55.5	57.9	70.0	60.4	90.6	88.3	101.9	95.8
70	61.0	54.9	53.9	51.7	68.6	51.7	88.9	87.4	101.4	94.1
80	58.5	53.7	53.2	52.1	65.7	51.7	86.7	85.3	101.3	92.3
Carrier Control	50.4	50.7	51.1	50.1	56.3	56.1	56.2	54.1	53.1	52.2

Data were analyzed statistically and calculated LSD value was 3.14.

P. oxalicum was found to retain its natural resistance induction features more efficiently in Bhagaas under normal room conditions (Figure 1). Reduction in the resistance induction abilities with passing time was the minimum in Bhagaas; while Saw Dust proved to be second most supporting carrier material among the five carriers checked. The steepest slope of sand revealed that it allowed fungal inoculum to work efficiently at first but with the passing time it was most difficult for *P. oxalicum* to maintain its efficacy in sand. So, it can definitely be said that Sand was the least supporting carrier material for *P. oxalicum* (Figure 1).

Table 4. Diameter of stems of tomato plants (cm) measured at 5 cm above from soil surface.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	1.43	1.41	1.68	1.69	1.80	1.78	1.88	1.88	1.89	1.88
10	1.43	1.42	1.59	1.54	1.76	1.71	1.85	1.83	1.88	1.88
20	1.40	1.41	1.54	1.47	1.65	1.62	1.81	1.80	1.84	1.87
30	1.37	1.39	1.43	1.34	1.63	1.58	1.75	1.76	1.85	1.86
40	1.32	1.33	1.42	1.31	1.56	1.51	1.73	1.70	1.82	1.83
50	1.21	1.25	1.34	1.24	1.48	1.46	1.67	1.65	1.79	1.81
60	1.16	1.19	1.26	1.19	1.35	1.37	1.62	1.59	1.78	1.80
70	1.03	1.11	1.11	1.07	1.27	1.30	1.57	1.54	1.75	1.78
80	0.98	1.00	0.92	0.91	1.23	1.22	1.56	1.51	1.75	1.78
Carrier Control	0.95	0.96	0.90	0.90	0.91	0.91	0.91	0.90	0.91	0.90

Data were analyzed statistically and calculated LSD value was 0.034.

Table 5. Number of leaves per tomato plant after application of *Penicillium oxalicum* in different carrier materials with various incubation periods.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	144	139	161	155	175	180	190	184	198	189
10	144	134	152	146	153	173	190	195	191	196
20	137	135	141	139	148	161	187	181	198	198
30	135	127	137	131	126	147	190	175	195	188
40	132	121	127	114	122	145	183	183	196	179
50	124	109	106	107	119	129	184	182	182	182
60	121	118	106	92	107	111	173	172	187	181
70	115	112	99	87	109	85	170	166	185	176
80	112	107	94	89	80	78	163	160	181	177
Carrier Control	97	99	79	71	76	76	76	73	71	75

Data were analyzed statistically and calculated LSD value was 3.92.

Table 6. Number of fruits per plant, under activities of *Penicillium oxalicum* and carrier materials.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	89	87	98	99	110	109	124	121	127	122
10	86	83	95	91	86	91	126	119	125	124
20	85	85	87	90	75	79	121	118	126	122
30	81	80	74	86	68	61	118	107	124	121
40	72	75	76	78	51	52	117	105	120	118
50	70	74	65	69	38	35	111	101	118	120
60	66	68	61	64	31	31	109	97	120	118
70	65	65	57	57	32	34	105	98	117	115
80	65	64	52	55	30	32	101	95	116	113
Carrier Control	47	49	32	31	30	33	34	31	30	29

Data were analyzed statistically and calculated LSD value was 2.13.

Average of grades obtained by individual carrier materials in individual Agro-economic aspects, depicted Bhagaas as the best carrier material because it maximum supported *P. oxalicum* inoculum for boosting 7 out of out 8 quality parameters of tomatoes. The only parameter to which Bhagaas stood second in support was "number of leaf hairs". Leaf hairs were insignificantly most dense in case of Saw Dust which was frequently proved second in supporting potential to *P. oxalicum* (Figure 2). Number of fruits was 66.1% more than any other treatment when tomatoes were treated with Bhagaas based inoculum. Moreover, efficiency of Bhagaas was significantly higher in terms of plant height and number of branches.

Table 7. Number of branches of tomato plants treated with *Penicillium oxalicum* in various carrier materials.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	25	24	30	29	31	30	32	34	35	35
10	25	24	30	31	27	25	34	35	34	34
20	26	24	27	25	22	26	33	34	35	36
30	24	23	28	26	19	21	31	33	35	34
40	23	27	24	21	17	16	29	30	34	35
50	22	21	21	18	16	15	30	28	35	36
60	23	23	19	19	15	15	26	27	34	34
70	22	24	16	15	16	15	27	24	33	33
80	21	22	16	16	16	16	26	25	33	31
Carrier Control	18	19	15	16	15	15	16	16	15	16

Data were analyzed statistically and calculated LSD value was 2.21.

Table 8. Average weight of five fruits (g), randomly selected from tomato plants having treatment combinations of carrier materials and incubation periods with *Penicillium oxalicum.*

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	193	201	205	198	206	204	201	201	205	206
10	193	204	196	203	195	203	207	196	203	204
20	198	196	198	184	192	184	194	194	201	196
30	188	183	193	182	173	140	186	173	195	195
40	173	170	174	162	150	143	183	171	198	181
50	157	162	171	162	144	124	188	172	196	183
60	142	155	155	143	149	121	175	164	189	177
70	141	148	157	141	134	134	173	155	181	170
80	145	125	137	101	112	97	167	153	178	169
Carrier Control	102	93	51	52	49	43	53	50	55	56

Data were analyzed statistically and calculated LSD value was 3.88.

Table 9. Chlorophyll contents (mg/g) in the leaves of *Penicillium oxalicum* treated tomato plants.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	1.05	1.01	1.15	1.12	1.21	1.21	1.29	1.31	1.30	1.29
10	1.05	0.97	1.10	1.03	1.05	1.02	1.26	1.27	1.30	1.31
20	1.01	0.96	1.01	0.94	0.94	0.83	1.27	1.21	1.31	1.29
30	0.97	0.94	0.93	0.81	0.79	0.61	1.21	1.18	1.26	1.29
40	0.94	0.89	0.78	0.70	0.58	0.47	1.24	1.15	1.27	1.27
50	0.95	0.85	0.71	0.59	0.39	0.32	1.16	1.14	1.24	1.27
60	0.87	0.85	0.61	0.54	0.37	0.33	1.14	1.09	1.25	1.28
70	0.85	0.81	0.52	0.41	0.34	0.31	1.09	1.06	1.24	1.27
80	0.80	0.78	0.35	0.31	0.34	0.30	1.04	1.06	1.21	1.26
Carrier Control	0.72	0.69	0.31	0.33	0.28	0.29	0.31	0.28	0.33	0.31

Data were analyzed statistically and calculated LSD value was 0.042.

Table 10. Number of leaf hairs per 9mm2 leaf area after application of *Penicillium oxalicum*.

Incubation days	Talc		Bentonite		Sand		Saw dust		Bhagaas	
	R	S	R	S	R	S	R	S	R	S
0	13	14	17	17	21	20	25	26	26	24
10	13	14	15	14	19	16	25	25	25	26
20	13	14	16	15	13	11	26	26	24	26
30	14	13	14	13	8	9	25	24	25	25
40	13	14	13	11	7	6	23	25	23	24
50	14	12	11	9	5	4	24	25	24	22
60	12	13	8	6	3	4	24	23	22	23
70	11	13	6	5	4	4	23	21	22	21
80	10	11	3	4	3	3	22	20	21	21
Carrier Control	3	4	5	3	4	3	4	4	3	4

Data were analyzed statistically and calculated LSD value was 0.96.

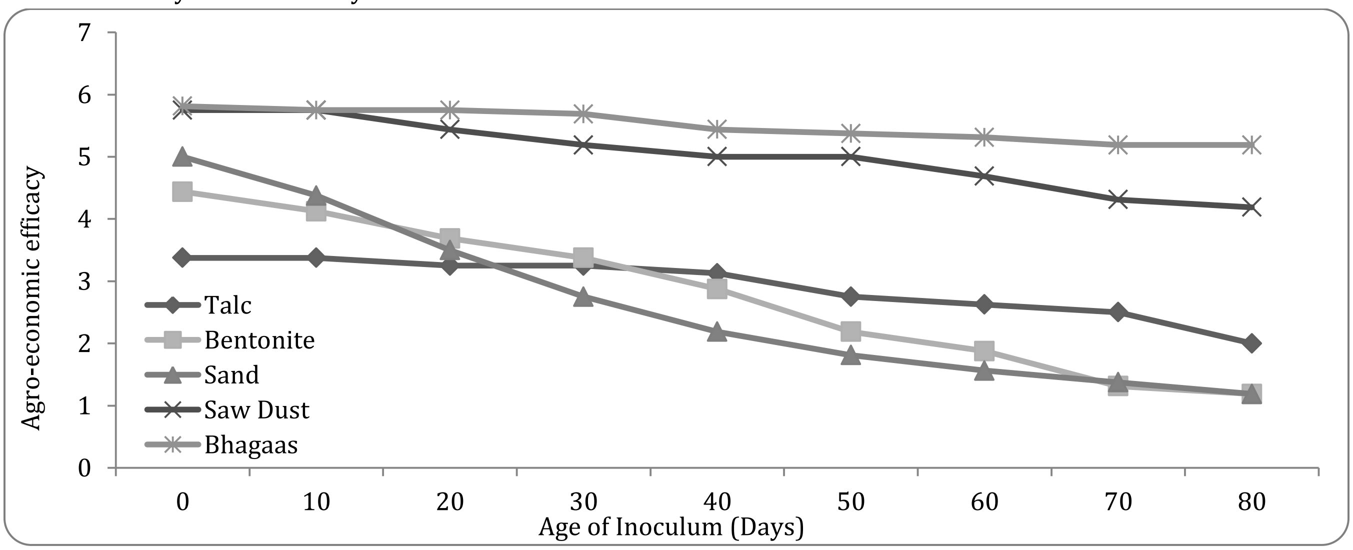

Figure 1: Efficacy of carrier materials in support to *Penicillium oxalicum* resistance induction potential. Minimum slope represents maximum support to *P. oxalicum* by carrier material. While, the steepest slope denote highest reduction in resistance induction potential of fungal species with passing time.

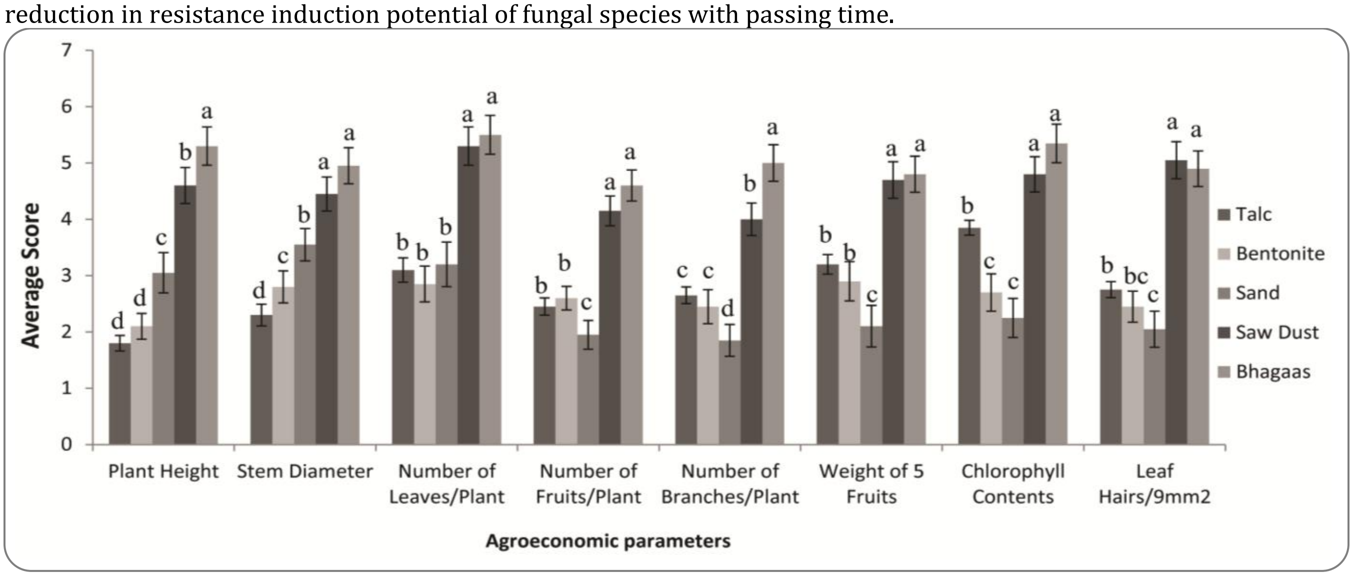

Figure 2. Response of different carrier materials and *P. oxalicum* for enhancing Agro-economic characters in tomatoes.

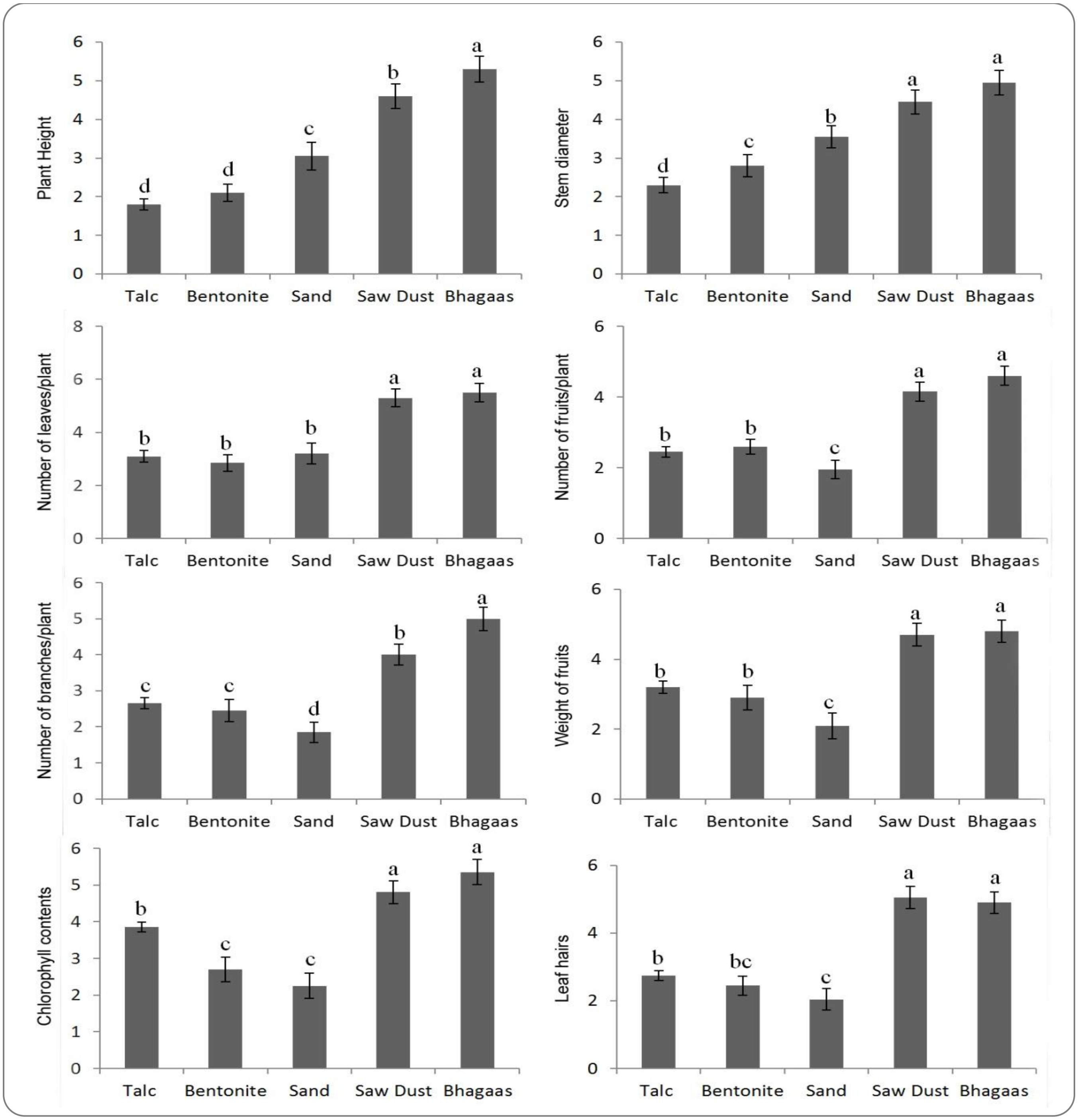

Figure 3: Effect of different carrier materials towards resistance induction potential of *P. oxalicum* in tomatoes against *A. alternata*. Agro-economic parameters studied are Plant height (A); Stem diameter (B); Number of leaves/ plant (C); Number of fruits/ plant (D); Number of branches/ plant (E); Weight of fruits (F); Chlorophyll contents (G); Leaf hairs (H).

Whereas, stem diameter, weight of fruits, chlorophyll contents and number of leaves and fruits per plant were recorded insignificantly greater with Bhagaas in comparison to saw dust treatment (Figure 2). Remaining three carrier materials were inappropriate because of exhibiting least efficiency in one or more agro-economic parameters and none of those three provided best response in any parameter investigated. Talc treatments provided least plant height and stem diameters; while, bentonite treatments were the poorest with reference to number of leaves and fruits per plant. Sand got minimum grades during the measurement of number of branches, weight of fruits, chlorophyll contents and density of leaf hairs (Figure 2).

Overall average of all grades in Figure 2 constructed an overall efficiency index of carrier materials which has been demonstrated in Figure 3. It proved Bhagaas as significantly the best carrier for *P. oxalicum* closely followed by Saw Dust (Figure 3). Talc could be denoted as third among the list of carrier materials with insignificantly superior performance than Sand. While the least carrying potential for *P. oxalicum* was of Sand; which had significant difference with Talc but non-significant difference with Bentonite (Figure 3).

DISCUSSION

Change in fungal culture viability with varying carrier materials is a well-known and frequently investigated phenomenon (Bakerspiegel, 1953; D'amelio *et al*., 2007; Agarwal *et al*., 2012). So, best suitable carrier materials have always been a topic of interest to maintain viability of fungal inoculums for numerous preservation and research related purposes. Therefore, previous studies exactly explain the varying disease control behavior of *P. oxalicum* inoculums prepared in different carrier materials as they affect viability of fungal propagules. Another additional fact is that altering preservation conditions may not only reduce viable fungal spore count but it may also result into undesired deviations in fungal characteristics of interest (Espinel-Ingroff *et al*., 2004). Hence it can easily be demonstrated that how the inoculum of single fungal species produced different disease control efficiencies when its inoculum in different carrier materials was applied to tomato plants. This study is a logical reminiscent of numerous previous researches in which the best supportive carrier materials have been investigated to maintain required fungal characteristics.

Variations in physical preservation conditions have a major role in defining features of recovered fungal species after incubation (Carmichael, 1962). Incubation period of fungal cultures in carrier materials for which they can maintain fungal characteristics intact is the most important factor in evaluating its preservation efficacy towards fungal species because different carrier materials have different viable incubation periods for a single fungal species (Dias *et al*., 1999). Similarly, in present investigation, five carrier materials based fungal inoculums were prepared and incubated for different periods at room temperature to evaluate carrier material which would maintain *Penicillium* characteristics intact for the longest incubation period. Therefore, this study falls on the pattern similar to previous studies.

Bhagaas was associated with decreased number of leaf hairs proving less plant resistance against insect pests (Rahman, 2002; Alexander *et al*., 2004). However, saw dust was noticed to produce more number of leaf hairs. Therefore, saw dust based formulations should be recommended for the areas under severe insect attacks. Sometimes, carrier materials have themselves a disease controlling potential (similar to fungal biocontrol agent) because plants may respond in such a way towards external chemical stimuli (Sharma *et al*., 2011). It was the reason behind disease controlling phenomenon carrier control treatment of Talc described in present research. It was also noticeable that resistance inducer made improvements in plant vigor index and Agro-economic aspects of tomato plants (Raja *et al.,* 2003; Madhaiyan *et al*., 2004; Islam *et al*., 2011).

Results of the present study conclude that Bhagaas and saw dust are the best carrier material to develop *P. oxalicum* formulation as a biological control agent; however, saw dust based formulations would be the best option for agriculture fields under severe insect attacks.

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