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Studies on the Efficacy and Management of Seed Borne Diseases and its Economics in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Seed borne diseases are regarded as major constraints in chickpea production. Seed diseases are caused by biotic factor like fungi and abiotic especially by drought, resulted in decrease in seed yield and productivity. Hence studies on the efficacy and management of seed borne diseases and its economics of seed production in chickpea was attempted.

Methods: The field experiment was carried out in Regional Agricultural Research Station, College of Agriculture, Vijayapur during *rabi* 2021-2022. The field experiment consisted eight treatments of fungicides and bioagents and combi- product of fungicide and bioagents *viz.*, T_1 : Carboxin 37% + thiram 37% DS @ 2 g/kg of seeds, T_2 : Carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed, T_3 : Thiophanatemethyl 45% + pyraclostrobin 5% FS @ 4 ml /kg of seeds, T_4 : Seed treatment with *Pseudomonas fluorescens* @ 10/kg of seed, T_5 : Seed treatment with *Trichoderma harzianum* @ 10/kg of seed, T_6 : Seed treatment with *Bacillus thuringenesis* @ 10/kg of seed, T_7 : Seed treatment with *Trichoderma harzianum* @ 10/kg of seed + carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed T_8 : Control (without seed treatment) were used to study the efficacy of treatments on growth yield and economics and per cent incidence against *R. bataticola* in field condition.

Result: Among the seed treatments carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed recorded significantly higher growth, seed yield, seed quality parameters and reduced per cent disease incidence and higher economics followed bythiophanatemethy 45% + pyraclostrobin 5% FS @ 4 ml/kg of seeds, as compared to control.

Key words: Bioagents, Economics of seed production, Fungicide, Growth parameters, Seed quality.

INTRODUCTION

Pulses are important constituents of Indian diet and supply major part in protein requirement. India is the largest producer and also consumer of pulses in the world and it is a cheaper source of protein to overcome malnutrition among human beings. Pulses contain high percentage of quality protein nearly three times as much as cereals (Uma Devi and Ganesan, 2007). Globally recognized Desi and Kabuli types chickpea is a member of Fabaceae family and it is a cool season domesticated crop. The entire surface of the plant shoot, except the corolla, is densely covered with fine hairs known as trichomes, many are glandular and secrete a highly acidic substance containing malic, oxalic and citric acids. These acids play an important role in protecting the plant against insect-pests. Chickpea accounts for more than 20% of global pulse production with a productivity of 1192 kg/ha, India leads the globe in chickpea area (9.99 million ha) and production (11.97 million tonnes). With an area of (7.13 lakh ha) and an annual production of 4.45 lakh tonnes, Karnataka is India's fourth largest producer of chickpeas, with an average productivity of 625 kg/ha (Anonymous, 2021). Chickpeas are widely grown in Northern Karnataka. The pathogens are inevitable guest menacing at different growth stages starting from emerging seedling stages to harvesting and continue till post-harvest interlude. They affect seed storability, physical appearance, viability and germinations. The proper identifications of these pathogens, their symptomatological appearance on the affected plants and their host pathogen relationship will

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definitely help us to formulate management strategies effectively that will ultimately increase the yield and better of pulses. Seed borne diseases are regarded as major constraints in chickpea production. Infected seeds serve as the source for the spread of the pathogen in disease free area. Seed infection affects the import and export adversely because the seed affected with microbes is not acceptable in international market. Infected seeds fail to germinate or seedlings and plants grown from infected seeds in the field may avoid early infection, but at the later stage of growth they may also be affected (Gupta, 2006). In addition, pathogens may spread over a long distance and the seeds in which various pathogens are present will invade

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uninfected fields (Fakir et al., 2001). The seed treatment with bioagents is safe, economical, eco-friendly, cheap, can be done easily with locally available materials and non-harmful to seed, animals and human beings and seed treatment with fungicide has help to protect seeds from mycoflora not only in storage but also to protect the germinating seedling and promote good establishment. Therefore an attempt has been made to study the efficacy and management of seed borne diseases and economics in chickpea.

MATERIALS AND METHODS

The field experiment was carried out in Regional Agricultural Research Station, College of Agriculture, Vijayapura, Karntaka state during rabi 2021-2022 to study the efficacy and management of seed borne disease and economics of seed production in chickpea in field. Eight treatments of fungicides and bioagents and combi-product of fungicide and bioagents viz.,T₁: Carboxin 37% + thiram 37% DS @ 2 g/kg of seeds, T₂: Carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed, T₃: Thiophanatemethyl 45% + pyraclostrobin 5% FS @ 4 ml /kg of seeds, T4: Seed treatment with Pseudomonas fluorescens @ 10/kg of seed, T₅: Seed treatment with *Trichoderma harzianum*@ 10/kg of seed, T6: Seed treatment with Bacillus thuringenesis @ 10/kg of seed, Tz: Seed treatment with Trichoderma harzianum @ 10/kg of seed + carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed T₈: Control (without seed treatment) were used. Observations on seed quality parameters on seed growth and yield parameters, disease incidence along with biochemical parameters and economics parameters were recorded (Anonymous, 2019).

RESULTS AND DISCUSSION

The results on seed yield and yield attributing parameters are presented in Table 1 and 2. Seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed (T_a) recorded significantly higher seed yield (1103 kg/ha) followed by T₃ (1083 kg/ha), T₁ (1070 kg/ha), T₇ (1010 kg/ha) as compared to control (811 kg/ha). Treatment carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed (T₂) recorded an higher increase in the yield to the extent of (36.00%) followed by T_3 (33.53%), T_1 (31.93%), T_7 (24.52%) respectively over control and yield parameters these parameters where shown higher by seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed (T₂) with various parameter i.e., seed yield/plant (7.61 g), seed yield/ plot (1.02 kg) increase in yield was due to increase in number of pods/plant (60.73), seed yield/plant (8.21 g), seed yield/plot (1.04 kg) this treatment was followed by (T₃) thiophanate methyl 45% + pyraclostrobin 5% FS @ 4 ml per kg of seeds where number of pods/plant (59.88). Increased in yield parameters may be due to seed treatment which improves seed germination, uniform plant stand and exposure of these plants for proper harnessing of sunlight, photosynthesis and its translocation of food metabolites to different growing parts and leading to better vegetative growth resultung increased number of primary and secondary branches per plant which add together for production of more number of flowers, pods, seeds per pod and test weight of seed leading to higher seed yield per plant and per ha (Shinde and Hunje, 2020, Rasool et al., 2015). The results are in conformity with findings of Durga et al. (2013) in chickpea.

Table 1: Effect of fungicides and bioagents on growth parameters in chickpea seeds.

Treatments	Number of	Number of	Plant height at	Days to	Days to	
	primary branches	secondary branches	harvest (cm)	50% flowering	maturity	
T ₁	2.3	13.83	35.10	48.33	98.33	
T ₂	2.6	15.16	38.20	47.66	97.33	
T ₃	2.6	14.46	36.13	48.33	98.33	
T ₄	2.2	12.08	32.79	49.33	99.33	
T ₅	2.3	12.63	33.67	49.00	99.33	
T ₆	2.2	11.83	32.47	49.33	99.66	
T ₇	2.3	13.26	33.72	48.66	99.00	
T ₈	2.1	11.40	31.34	49.33	99.66	
Mean	2.34	13.09	34.18	48.75	98.87	
S.Em. ±	0.13	0.40	1.27	1.38	3.10	
C.D. @ 5%	NS	1.22	3.86	NS	NS	

Note: T_1 : Seed treatment with carboxin 37.5% + thiram 37.5% DS @ 2 g/kg of seeds.

T₂: Seed treatment with carbendazium 25% + mancozeb 50% WS @ 3.5 g/kg of seeds.

T₃: Seed treatment with thiophanate methyl 45% + pyraclostrobin 5% FS @ 4 ml/kg of seeds.

 $[\]rm T_{\rm 4}\!\!:$ Seed treatment with Pseudomonas fluorescens @ 10 g/kg of seeds.

T₅: Seed treatment with *Trichoderma harzianum* @ 10 g/kg of seeds.

 T_6 : Seed treatment with Bacillus thuringiensis @ 10 g/kg of seeds.

T₇: Seed treatment with *Trichoderma harzianum* @ 10 g and carbendazium 25% + mancozeb 50% WS @ 3.5 g/kg of seeds.

T_s: Control (Without seed treatment).

Seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed (Table 1) recorded higher number of primary branches (2.66), secondary branches (15.16), plant height (38.20), early days to 50% flowering (47.33) and early days to maturity (97.33) as compared to control. The seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed which is a contact and systematic fungicide which mainly helps in protection of seed. Chickpea seeds have very soft, delicate seed coat that are sensitive and prone to damage by both biotic and abiotic factors from invading and survival of wide range of fungal pathogens around rhizosphere throughout the crop period and provides a favorable condition by enhancing sufficient nutrient uptake from rhizosphere through better root system. Shinde et al. (2020). These results are in conformation with the findings of Anitha et al. (2013) in Soybean and Xalxo et al. (2007) in chickpea.

Seed treatment with fungicides and bioagents enhanced the seed quality parameters significantly as compared to control (Table 3). Seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed (T₂) showed higher seedling vigour index I (2612), seedling vigour index II (27935) due to higher in germination percentage (97.33%), root length (18.26 cm), shoot length (8.56 cm), seedling dry weight (287 mg), followed by (T₃) thiophanate methyl 45% + pyraclostrobin 5% FS @ 4 ml per kg of seeds is due to increased the rate of imbibitions. The fungicidal seed treatment on the surface act as moisture attracting material and improve germination due to proper supply of water and nutrients and reflected by reducing the seed infection by pathogen and due to induction of photosynthesis and

synthesis of α -amylase, protease and other hydrolytic enzymes which appear to induce the activity of gluconeogenic enzymes during early stage of seed germination cause lengthy seedling and also higher 100 seed weight directly correlated with the higher seedling (Rajendraprasadet al., 2021 and Biabani Katozi, 2011) and Lower electric conductivity was shown by seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seed (T_a) with (0.722) and higher protein content (22%). The lower electrical conductivity was recorded in treated seeds over untreated seeds which may be due to fungicide that protects the seeds from storage pathogens and thus reduces the seed infection, cracks and aberrations of the seed coat and also the leaching of electrolytes. These results are in agreement with Maheshbabu and Ravi Hunje (2008) in soybean.

The data on seed treatment with carbendazim $25\% + \text{mancozeb}\ 50\%\ WS\ @\ 3.5\ g/kg$ of seed (T_2) presented in Table 2 and showed significantly lesser disease incidence (8.90%) from rest of the treatment. The next best treatment was seed treatment with thiophanate methyl $45\% + \text{pyraclostrobin}\ 5\%\ FS\ @\ 4\ ml\ per\ kg\ of\ seeds\ T_3\ recorded\ (10.48\%)$ as compared to control. The T_8 recorded (28.66%) of disease incidence due to mixture of this fungicide in which mancozeb is contact action and preventive fungicide with multisite mode of action and it remains on seed surface in treated seeds. It is fungitoxic when exposed to air, converted into isothiocynate, which inactivates the sulphahydral group of enzymes in fungi, causing disturbance in fungal enzyme functioning. Where as carbendazim is systemic in action acts as preventive and curative. It also acts by disrupting

Table 2: Effect of fungicides and bioagents on seed yield parameters and disease incidence in chickpea seeds.

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	Number	Number	Seed	Seed	Seed	Disease	Reduction
Treatments	of pods/	of seeds/	yield	yield	yield	incidence	over
	plants	pods	(g/plant)	(kg/plot)	(kg/ha)	(%)	control (%)
T ₁	58.28	1.13	7.51	1.01	1070	12.33 (20.55)	56.97
$T_{_2}$	60.73	1.20	8.21	1.04	1103	8.90 (17.32)	68.94
T ₃	59.88	1.13	7.61	1.02	1083	10.48 (18.87)	63.43
T ₄	53.51	1.06	6.87	0.91	966	18.50 (25.47)	35.45
T ₅	53.65	1.06	6.89	0.93	986	17.48 (24.70)	39.00
T ₆	52.20	1.00	6.74	0.86	907	20.60 (26.92)	28.12
T ₇	56.08	1.06	6.99	0.95	1010	13.73 (21.68)	52.09
T ₈	51.20	1.00	6.51	0.77	811	28.66 (32.35)	-
Mean	55.69	1.08	6.86	0.94	992	18.67 (26.83)	
S.Em±	1.95	0.06	0.33	0.05	56.12	0.84	
C.D. @ 5%	5.91	NS	1.00	0.16	170.22	2.61	

Note: T1: Seed treatment with carboxin 37.5% + thiram 37.5% DS @ 2 g/kg of seeds.

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 $[\]rm T_2\!:$ Seed treatment with carbendazium 25% + mancozeb 50% WS @ 3.5 g/kg of seeds

T₃: Seed treatment with thiophanate methyl 45% + pyraclostrobin 5% FS @ 4 ml/kg of seeds.

T₄: Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seeds.

T₅: Seed treatment with *Trichoderma harzianum* @ 10 g/kg of seeds.

 T_6 : Seed treatment with *Bacillus thuringiensis*@ 10 g/kg of seeds.

T₇: Seed treatment with *Trichoderma harzianum* @ 10 g and Carbendazium 25% + Mancozeb 50% WS @ 3.5 g/kg of seeds.

T_s: Control (Without seed treatment).

the spindle formation during cell division in fungi. (Sharma et al., 2017).

The data on economics in chickpea seed production was significantly influenced by seed treatment are presented in Table 4. Significantly higher gross returns, net returns, B:C were recorded in the treatment with carbendazim 25%

+ mancozeb 50 % WS @ 3.5 g/kg of seed (T_2) (` 60,647, ` 37,964 and 2.67 respectively) followed by seed treatment with thiophanate methyl 45% + pyraclostrobin 5% FS @ 4 ml per kg of seeds (` 59,583, ` 36,491, 2.58). The gross returns are governed by higher seed yield fetching better price. Due to seed treatment with fungicides and biocontrol agent,

Table 3: Effect of fungicides and bioagents on seed quality and biochemical parameters in chickpea seeds.

Test	Seed	Root	Shoot	Seedling	Seedling	Seedling	Seedling	Electrical	Protein
weight	germination	length	length	length	dry weight	vigour	vigour	conductivity	content
(g)	(%)	(cm)	(cm)	(cm)	(mg)	index I	index II	(dS/m)	(%)
22.96	95.67 (78.06)*	15.03	7.80	22.83	282	2184	27042	0.738	22.11
23.94	97.33 (80.64)	18.26	8.56	26.83	287	2612	27935	0.722	22.39
23.84	96.33 (78.98)	16.73	8.03	24.77	283	2385	27326	0.730	22.18
21.92	93.00 (74.68)	13.36	5.90	19.27	268	1791	24954	0.760	22.01
22.32	93.67 (75.43)	14.10	6.76	20.87	271	1954	25413	0.755	22.03
21.40	90.67 (72.25)	12.73	5.46	18.20	259	1651	23479	0.765	21.73
22.71	95.33 (77.54)	14.80	7.00	21.80	277	2078	26440	0.742	22.10
20.67	87.67 (69.44)	11.86	5.36	17.23	247	1510	21713	0.783	21.39
22.47	93.71	14.61	6.86	21.47	272	2021	25537	0.728	21.99
0.63	0.54	0.54	0.32	0.66	2.35	65.4	240	0.014	0.29
2.66	2.26	2.24	1.32	2.73	9.72	270	993	NS	NS
	weight (g) 22.96 23.94 23.84 21.92 22.32 21.40 22.71 20.67 22.47 0.63	weight (g) germination (%) 22.96 95.67 (78.06)* 23.94 97.33 (80.64) 23.84 96.33 (78.98) 21.92 93.00 (74.68) 22.32 93.67 (75.43) 21.40 90.67 (72.25) 22.71 95.33 (77.54) 20.67 87.67 (69.44) 22.47 93.71 0.63 0.54	weight (g) germination (%) length (cm) 22.96 95.67 (78.06)* 15.03 23.94 97.33 (80.64) 18.26 23.84 96.33 (78.98) 16.73 21.92 93.00 (74.68) 13.36 22.32 93.67 (75.43) 14.10 21.40 90.67 (72.25) 12.73 22.71 95.33 (77.54) 14.80 20.67 87.67 (69.44) 11.86 22.47 93.71 14.61 0.63 0.54 0.54	weight (g) germination (cm) length (cm) length (cm) 22.96 95.67 (78.06)* 15.03 7.80 23.94 97.33 (80.64) 18.26 8.56 23.84 96.33 (78.98) 16.73 8.03 21.92 93.00 (74.68) 13.36 5.90 22.32 93.67 (75.43) 14.10 6.76 21.40 90.67 (72.25) 12.73 5.46 22.71 95.33 (77.54) 14.80 7.00 20.67 87.67 (69.44) 11.86 5.36 22.47 93.71 14.61 6.86 0.63 0.54 0.54 0.32	weight (g) germination (%) length (cm) length (cm) length (cm) 22.96 95.67 (78.06)* 15.03 7.80 22.83 23.94 97.33 (80.64) 18.26 8.56 26.83 23.84 96.33 (78.98) 16.73 8.03 24.77 21.92 93.00 (74.68) 13.36 5.90 19.27 22.32 93.67 (75.43) 14.10 6.76 20.87 21.40 90.67 (72.25) 12.73 5.46 18.20 22.71 95.33 (77.54) 14.80 7.00 21.80 20.67 87.67 (69.44) 11.86 5.36 17.23 22.47 93.71 14.61 6.86 21.47 0.63 0.54 0.54 0.32 0.66	weight (g) germination (%) length (cm) length (cm) length (cm) length (cm) dry weight (mg) 22.96 95.67 (78.06)* 15.03 7.80 22.83 282 23.94 97.33 (80.64) 18.26 8.56 26.83 287 23.84 96.33 (78.98) 16.73 8.03 24.77 283 21.92 93.00 (74.68) 13.36 5.90 19.27 268 22.32 93.67 (75.43) 14.10 6.76 20.87 271 21.40 90.67 (72.25) 12.73 5.46 18.20 259 22.71 95.33 (77.54) 14.80 7.00 21.80 277 20.67 87.67 (69.44) 11.86 5.36 17.23 247 22.47 93.71 14.61 6.86 21.47 272 0.63 0.54 0.54 0.32 0.66 2.35	weight (g) germination (%) length (cm) length (cm) length (cm) dry weight (mg) vigour index I 22.96 95.67 (78.06)* 15.03 7.80 22.83 282 2184 23.94 97.33 (80.64) 18.26 8.56 26.83 287 2612 23.84 96.33 (78.98) 16.73 8.03 24.77 283 2385 21.92 93.00 (74.68) 13.36 5.90 19.27 268 1791 22.32 93.67 (75.43) 14.10 6.76 20.87 271 1954 21.40 90.67 (72.25) 12.73 5.46 18.20 259 1651 22.71 95.33 (77.54) 14.80 7.00 21.80 277 2078 20.67 87.67 (69.44) 11.86 5.36 17.23 247 1510 22.47 93.71 14.61 6.86 21.47 272 2021 0.63 0.54 0.54 0.32 0.66 2.35 65.4	weight (g) germination (%) length (cm) length (cm) length (cm) dry weight (mg) vigour index I vigour index II 22.96 95.67 (78.06)* 15.03 7.80 22.83 282 2184 27042 23.94 97.33 (80.64) 18.26 8.56 26.83 287 2612 27935 23.84 96.33 (78.98) 16.73 8.03 24.77 283 2385 27326 21.92 93.00 (74.68) 13.36 5.90 19.27 268 1791 24954 22.32 93.67 (75.43) 14.10 6.76 20.87 271 1954 25413 21.40 90.67 (72.25) 12.73 5.46 18.20 259 1651 23479 22.71 95.33 (77.54) 14.80 7.00 21.80 277 2078 26440 20.67 87.67 (69.44) 11.86 5.36 17.23 247 1510 21713 22.47 93.71 14.61 6.86 21.47	weight (g) germination (%) length (cm) length (cm) dry weight (mg) vigour index I vigour index II conductivity (dS/m) 22.96 95.67 (78.06)* 15.03 7.80 22.83 282 2184 27042 0.738 23.94 97.33 (80.64) 18.26 8.56 26.83 287 2612 27935 0.722 23.84 96.33 (78.98) 16.73 8.03 24.77 283 2385 27326 0.730 21.92 93.00 (74.68) 13.36 5.90 19.27 268 1791 24954 0.760 22.32 93.67 (75.43) 14.10 6.76 20.87 271 1954 25413 0.755 21.40 90.67 (72.25) 12.73 5.46 18.20 259 1651 23479 0.765 22.71 95.33 (77.54) 14.80 7.00 21.80 277 2078 26440 0.742 20.67 87.67 (69.44) 11.86 5.36 17.23 247

Note: *Figures in the parentheses are arcsine transformed value.

NS-Non significant.

Table 4: Effect of fungicides and bioagents on benefit cost ratio in chickpea.

Treatments	Cost of cultivation (`per ha)	Gross return (` per ha)	Net return (` per ha)	B:C
T ₁	22700	58850	36150	2.59
T_2	22683	60647	37964	2.67
T ₃	23092	59583	36491	2.58
$T_{_4}$	22564	53130	30566	2.35
T ₅	22564	54248	31684	2.40
T ₆	22564	49903	27339	2.21
T ₇	22715	55550	32835	2.45
T ₈	22532	44605	22073	1.98
Mean	-	54564	31887	2.41
S.Em. ±	-	3086	3086	0.14
C.D. @ 5%	-	9362	9362	0.41

Note: T₁: Seed treatment with Carboxin 37.5% + Thiram 37.5% DS @ 2 g/kg of seeds.

T₁: Seed treatment with carboxin 37.5% + thiram 37.5% DS @ 2 g/kg of seeds.

T₂: Seed treatment with carbendazium 25% + mancozeb 50% WS @ 3.5 g/kg of seeds.

T₃: Seed treatment with thiophanate methyl 45% + pyraclostrobin 5% FS @ 4 ml/kg of seeds .

T₄: Seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seeds.

T₅: Seed treatment with *Trichodermaharzianum* @ 10 g/kg of seeds.

 T_6 : Seed treatment with *Bacillus thuringiensis* @ 10 g/kg of seeds.

T₇: Seed treatment with *Trichoderma harzianum* @ 10 g and carbendazium 25% + mancozeb 50% WS @ 3.5 g/kg of seeds.

T_s: Control (Without seed treatment).

T₂: Seed treatment with Carbendazium 25% + Mancozeb 50% WS @ 3.5 g/kg of seeds.

 T_3 : Seed Treatment with Thiophanate methyl 45% + Pyraclostrobin 5% FS @ 4 ml/kg of seeds.

 T_4 : Seed Treatment with *Pseudomonas fluorescens* @ 10 g/kg of seeds.

T₅: Seed Treatment with *Trichodermaharzianum* @ 10 g/kg of seeds.

 T_6 : Seed Treatment with *Bacillus thuringiensis* @ 10 g/kg of seeds.

T₇: Seed Treatment with *Trichoderma harzianum* @ 10 g and Carbendazium 25% + Mancozeb 50% WS @ 3.5 g/kg of seeds.

T_s: Control (Without seed treatment).

superior seed yield was obtained. This resulted in higher gross returns and net returns there by increased benefit cost ratio as this treatment and increases the grain yield by reducing the mycoflora infestation. Similar results were noticed by Mohdet al. (2004).

CONCLUSION

It can be concluded that the seed treatment with carbendazim 25% + mancozeb 50% WS @ 3.5 g/kg of seeds have good effect on seed yield per ha, better seed quality, reduced diseases incidence and higher net return followed by T_3 thiophanatemethy I45% + pyraclostrobin 5% FS @ 4 ml/kg of seed compared to control.

Conflict of interest: None.

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