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Effect of bio-control agents treatments on Maize seed vigour by Standard methods

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ABSTRACT
Maize is one of the most important cereal crops in the world. This crop is subjected to as many as 112 diseases and among them, more than 70 are seed borne on a global basis. Fungal infection is the most widespread in stored grain and appears as mold or caking on the affected ear or grain. The biocontrol management not only preserve the surrounding but also reduces the potential for air and ground water contamination, maintains or increases the cost-effectiveness of disease management programs and reduces or eliminates issues related to pesticide residue. Therefore, the seed treatments were done with two bio-control agents, Trichoderma harzianumand Trichoderma asperellum. The seed quality was measured as by nine parameters was recorded by multi-pots tray and paper rolled method. In case of original seed treatments with Trichoderma harzianum(Th) showed range of per cent germination 67.00±18.58 (V8) to 96.00±3.27 (V4), germination index (seed per day) 5.11±1.00 (V2) to 7.25±0.94 (V1), accumulative speed (seed per day) 10.08±2.23 (V2) to 15.80±1.73 (V1), coefficient of germination rate (%) 7.85±1.89 (V10) to 17.95±1.45 (V8)], fresh weight (g) 9.85±2.73 (V10) to 15.67±1.15 (V7), dry weight (g) 2.37±0.24 (V5) to 4.03±0.29 (V7), seedling length (mm) 195.62±35.18 (V5) to 297.50±20.62 (V2), seed vigour index–seedling length 15765.77±2546.64 (V5) to 25275.00±7646.67 (V7), and seed vigour index–dry weight 179.45±100.27 (V10) to 380.02±55.79 (V7) and Trichoderma asperellum (Ta) seed treatment was achieved highest values of per cent germination 92.00±8.64 (V7), germination index (seed per day) 7.25±1.05 (V1), accumulative speed (seed per day) 16.37±2.44 (V1), coefficient of germination rate (%) 14.75±1.61 (V11), fresh weight (g) 15.33±1.44 (V7), dry weight (g) 3.94±0.37 (V7), seedling length (mm) 253.21±8.64 (V1), seed vigour index–seedling length 22608.62±3942.72 (V1) and seed vigour index–dry weight 364.94±66.23 (V7). In case paper rolled method of original seed treatment with harzianum (Th) and harzianum asperellum (Ta) excelled significant superior performance by contributing per cent germination 94.00±5.16 (V12) and 93.00±8.25 (V6), fresh weight (g) 12.25±1.26 (V1) and 13.00±0.82 (V3), dry weight (g) 3.68±0.38 (V1) and 3.90±0.24 (V3), seedling length (mm) 197.50±56.79 (V9) and 181.25±17.50 (V12), seedling vigour index- seedling length 18000.00±5275.15 (V9) and 16760.00±2475.05 (V6) and seedling vigour index- dry weight 341.70±17.73 (V4) and 355.80±46.99 (V6).

Key words: *Maize,Trichoderma harzianum and Trichoderma asperellum, Germination and Seed vigour index*

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INTRODUCTION

Hypocrea/Trichoderma species are economically important because of the effect that some of them have on disease-causing fungi. This antifungal activity has been extensively documented and suggestions have been made that the bio-control effects exerted by these fungi may be based on one or more of three possible factors, namely antibiosis [1,2,3,4,5], mycoparasitism[6,7], and nutrient competition [8]. The volume edited by Harman and Kubicek[9] contains several papers that include comprehensive information about enzymes of *Hypocrea/Trichoderma* biological control and commercial applications. Green ascospore-

producing species of *Hypocrea/ Trichoderma* that have known antifungal activities are *H. virens*(*T. virens*)and *H. lixii*(*T. harzianum*). In addition, *T. harzianum* and *T. virens*are the active ingredients in the commercial preparations, TRICHODEX ™ and SoilGard™, respectively, used in biological control of various fungal diseases [10,11,12].

Many species have the ability to break down cellulosic materials through the production of cellulases. This ability has led to the commercial exploitation of some *Hypocrea/Trichoderma* species in production of enzymes used in manufacture of clothes washing detergent, animal feed and fuel [13,14,15]. Species of *Trichoderma* such as *T. koningii, T. viride*and *T. harzianum*produce a variety of antibiotic antifungal peptides called peptaibols that are capable of interacting with cell membranes [16].

Efforts made include breeding for disease resistant varieties, the use of chemicals in reducing seed borne pathogens and the use of biological control agents to reduce plant pathogens. Chemical methods involving seed treatment with fungicides have been employed to improve germination, vigour, crop establishment, crop stands, and yield. However, indiscriminate use of chemicals for controlling plant diseases has resulted in environmental pollution and health hazards [17]. Moreover, haphazard use of chemicals breaks down the natural ecological balance by killing beneficial soil microbes[18]. In some cases farmers in developing countries cannot afford high cost of chemical pesticides. As a result of these problems there is an increasing attention towards the development of safer methods of disease control. This has resulted in the development of bio-pesticides for the control of seed borne pathogens of food crops. *Trichoderma* spp. as biocontrol agent are effective have little or no adverse effect on the environment.

MATERIAL AND METHODS

Effect of seed treatments with bio-control agents- Two bio-control agents were selected and its effect on germination was studied by rolled paper towel method and multi-pot Tray method-

Maintenance of bio-control agent

Antagonism of local isolates of *Trichoderma* spp. formulations of bio-control agents will be tested.Stock culture will be maintained by sub culturing on Potato Dextrose Agar (PDA) at 25 ± 1°C.

Mass multiplication of *Trichoderma* spp.

For mass multiplication, potato broth will be prepared by autoclaving at 15 psi for 20 minutes. Culture of *Trichoderma* spp. will be prepared in potato broth in 250 ml conical flasks by inoculating 10 mm disc of 7 to 10 days old *Trichoderma* spp. culture under aseptic conditions in laminar air flow chamber and incubated at 25 ± 1^{O}C. The spore count of *Trichoderma* spp. (2 x 10^8 spore/ml) will be taken before mixing it with pre sterilised talc powder and the pH will be adjusted to 7.0 by adding 150g calcium carbonate/kg talc powder. 1 kg of sterilized talc powder and 10g of carboxyl methyl cellulose (CMC 1%) will be transferred in a sterilized container under aseptic conditions. After that 400-500 g bio-agent suspension will be added to it and mixed thoroughly. Then, this product will be used for seed priming.

Soil preparation

Soil were collected from the field area and mixed uniformly with FYM @ 200 g/kg of soil. Soil sterilization were done with formalin (40%) of 5 ml formalin diluted with 20 ml of water for 4 kg of soil. Treated soil was covered with polythene sheets and maintained as such for 7 days and then exposed for 7 days for aeration.

Multi-pot tray method [19]

Original seed of maize variety 100 (100= 25x 4R) seeds both untreated and treated with bio-agents, *Trichoderma* spp.talc based formulation @ 0.5 g/100g seed were sown in 4 replications in line in the sterilized soil (1-2 cm depth) @ 100 seeds/tray (12x13). Untreated seeds also served as a control for bio-agents treated seeds. Every day, Shoots rising above soil surface were counted as germinated. Final data were take on 5 DAS (as final reading) in each untreated and bio-agent treated seeds trays. Though germination testing remains the principle and Internationally accepted, criterion for seed viability but for high germination seed lots, germination test result may not provide enough information as to potential seed lot performance. Therefore, vigour status of seed lot becomes important and vigour testing necessary.

Rolled paper towel method [20]**-** Germination of original seed of each maize variety used in different experiments were studied by employing little modified of rolled paper towel method as described by International Seed Testing Association [21]. Hundred seeds (25 seeds/ replication) were used in each case. Two sheets of germination paper were wetted with distilled water and placed above butter paper used as a base for the paper, leaving adequate margin. Hundred seeds (25 seeds/ replication) both untreated and treated with bio-agent, *Trichoderma* spp. talc based formulation @ 0.5 g/100g seed were sown it evenly and roll. Untreated seeds also served as a control for treated seeds. The rolls were placed in incubator at 28°C. Five days after sowing (DAS), all the normal seedlings were counted all replications and categories. Data was taken as germination (%), fresh weight (g), dry weight (g), seedling length (mm), Seedling vigour index-I and Seedling vigour index-II.

Standard Germination Test (%)

One hundred seeds with four replications each of all the maize varieties were tested in the laboratory according to the Rules of International Seed Testing Association [22]. The final count of germination was recorded on 5th day and the number of normal seedlings was counted and expressed as per cent germination.

$G\ (\%) = [N_T \times 100]/N$

Where,

N_T : Proportion of germinated seeds in each treatment for the final measurement

N : Number of seeds used in bioassay

Germination/Sprouting index [23]**-**

The germination index (GI) is defined as:

GI (Seed per day) $= \frac{\sum Ti\ Ni}{S}$

Where,

Ti = number of days after sowing,

Ni = number of seeds germinated on day i, and

S = total number of seed planted

Speed of Accumulated germination (AS) [24]**:**

AS (Seed per day) = [N1/1 + N2/2 + N3/3 ……. + Nn/n]

Where,

N1, N2, N3, Nn: Cumulative number of seeds which germinate on time 1, 2, 3, ……,n

Coefficient of the rate of germination (CRG) [25]**:** The CRG gives an indication of the rapidity of germination-

CRG (%) $= \frac{N1 + N2 + N3 + \ldots..+ Nn].X\ 100}{S(N1 \times T1) + (N2 \times T2) + (N3 \times T3) + \ldots\ldots (Nn \times Tn)}$

Where,

N1: number of germinated seeds at time T1

N2: number of germinated seeds at time T2

Nn: number of germinated seeds at time Tn

Seedling Length (mm)

Normal germinating seedlings in four replications each of all the varieties were selected and the seedling length was measured in millimeter and average seedling length was calculated.

Seedling Fresh Weight (g)

Normal germinating seedlings in four replications each of all the varieties were selected in separate paper bags and the seedling fresh weight was measured in gram and average seedling fresh weight was calculated.

Seedling Dry Weight (g)

For dry weight determination, all replication of seedlings are removed and dried in 2-3 days in air. These seedlings were placed in separate paper bags and then transferred into oven at 50°C for 8 h four times. The average weight of all replications of germination seedlings was taken and seedling dry weight was expressed in grams.

Vigour Indices

Seedling vigour indices were calculated according to the formulae suggested by Abdul-Baki and Anderson [26]-

Vigour index-I=Standard germination (%) x Seedling length (mm)

Vigour index-II=Standard germination (%) x Seedling dry weight (g)

Data analysis
Data for seed health parameterswere summarized and analysed using SAS ver. 9.1 (SAS Institute Inc., Cary, NC, USA) within the framework of general linear models. Themeans were separated using LSD (p=0.05) to determine whether there were significant differences among the samples obtained from the different maize growing zones.

RESULT AND DISCUSSION
Effect of seed treatments with bio-control agents-Two bio-control agents were selected and its effect on germination was studied by rolled paper towel method and multi-pot Tray method.
Multi-pot tray method [19]
Data relating to effect of bio-control agent on three seed categories are presented in **Tables 1a, b.**Over all mean of all seed quality parameter of twelve varieties revealed that seed treatment with *Trichoderma harzianum* (Th) and *Trichoderma asperellum* (Ta) were significantly superior over control. Among the nine seed quality parameters *viz.* germination (GN), germination index (GI), accumulative speed (AS), coefficient of germination rate (CRG), fresh weight (FW), dry weight (DW), seedling length (mm), seedling vigour index- seedling length (SVSL) and seedling vigour index- dry weight (SVDW) was recorded. In case of original seed treatment with *Trichoderma harzianum* (Th) showed values range of per cent germination 67.00±18.58 (V8) to 96.00±3.27 (V4), germination index (seed per day) 5.11±1.00 (V2) to 7.25±0.94 (V1), accumulative speed (seed per day) 10.08±2.23 (V2) to 15.80±1.73 (V1), coefficient of germination rate (%) 7.85±1.89 (V10) to 17.95±1.45 (V8), fresh weight (g) 9.85±2.73 (V10) to 15.67±1.15 (V7), dry weight (g) 2.37±0.24 (V5) to 4.03±0.29 (V7), seedling length (mm) 195.62±35.18 (V5) to 297.50±20.62 (V2), seedling vigour index- seedling length 15765.77±2546.64 (V5) to 25275.00±7646.67 (V7) and seedling vigour index- dry weight 179.45±100.27 (V10) to 380.02±55.79 (V7) and *Trichoderma asperellum* (Ta) seed treatment was achieved highest values of per cent germination 92.00±8.64 (V7), germination index (Seed per day) 7.25±1.05 (V1), accumulative speed (Seed per day)16.37±2.44 (V1), coefficient of germination rate (%) 14.75±1.61 (V11), fresh weight (g) 15.33±1.44 (V7), dry weight (g) 3.94±0.37 (V7), seedling length (mm) 253.21±8.64 (V1), seedling vigour index- seedling length 22608.62±3942.72 (V1) and seedling vigour index- dry weight [364.94±66.23 (V7).

Table 1a: Effect of bio-agents treatments on original seeds of maize through multi-pot tray method

	Germination (%)			Germination Index (Seed per day)			Accumulative Speed (Seed per day)			Coefficient of germination rate (%)		
Varieties	C	Th	Ta	C	Th	Ta	C	Th	Ta	C	Th	Ta
V1	84.00± 7.30	88.00± 4.62*	89.00±1 2.81	7.00± 0.99	7.25± 0.94	7.25± 1.05	14.97± 2.54	15.80± 1.73	16.37± 2.44	4.73±0 .17	14.25± 2.03	14.30± 1.57
V2	67.00± 8.87	71.00± 6.83	77.00±6 .00	5.39± 0.82	5.11± 1.00	5.23± 0.51	11.02± 2.31	10.08± 2.23	10.10± 1.20	4.47±0 .41	14.75± 0.91	11.60± 1.34
V3	68.00± 5.66	79.00± 6.83	80.00±6 .53	5.51± 0.54	6.40± 1.11	6.16± 0.70	11.40± 1.88	13.39± 1.87	13.19± 2.07	4.52±0 .36	13.80± 1.14	11.60± 0.67
V4	52.00± 7.30	96.00± 3.27	78.00±5 .16	4.44± 1.13	6.48± 0.73	5.58± 0.27	9.14±2 .37	11.41± 2.47	12.13± 2.86	6.65±0 .62	14.40± 2.03	8.80±0 .85
V5	81.00± 6.00	81.00± 8.25	80.00±6 .53	6.23± 0.46	5.59± 0.87	5.52± 0.65	12.40± 1.85	10.70± 1.97	10.96± 1.83	5.28±0 .64	14.95± 0.62	14.55± 1.94
V6	79.00± 3.83	88.00± 9.80	90.00±9 .27	6.13± 0.39	6.03± 0.74	6.02± 0.92	12.30± 1.74	11.38± 1.54	11.46± 2.00	5.80±0 .79	17.45± 2.78	14.05± 1.91
V7	81.00± 6.00	94.00± 6.93	92.00±8 .64	6.23± 0.46	6.33± 0.51	6.46± 0.70	12.40± 1.85	11.60± 1.37	12.56± 1.77	11.60± 1.37	12.56± 1.77	12.40± 1.85
V8	78.00± 2.31	81.00± 8.25	92.00±3 .27	6.08± 0.34	5.59± 0.87	6.12± 0.34	12.25± 1.82	10.70± 1.97	11.56± 1.49	5.28±0 .64	17.95± 1.45	13.80± 1.43
V9	68.00± 3.27	80.00± 9.24	80.00±1 0.33	5.41± 0.36	5.30± 0.51	5.71± 1.05	10.92± 1.92	10.58± 1.83	11.63± 2.86	5.20±0 .70	14.60± 1.40	11.95± 0.55
V10	68.00± 10.83	67.00± 18.58	81.00±8 .25	4.74± 1.57	5.56± 1.96	5.56± 0.91	10.36± 4.18	11.80± 8.59	10.51± 4.14	2.05±0 .63	7.85±1 .89	8.05±3 .10
V11	82.00± 2.31	87.00± 10.52	86.00±6 .93	6.03± 0.40	5.64± 0.66	5.82± 0.64	12.45± 1.76	11.00± 1.96	11.26± 1.79	5.77±0 .76	16.45± 1.15	14.75± 1.61
V12	71.00± 6.00	91.00± 3.83	90.00±5 .16	5.44± 0.54	5.95± 0.43	6.15± 0.37	11.72± 1.71	11.57± 1.66	11.84± 1.20	6.03±0 .28	17.30± 1.19	12.15± 1.53

OAM	**73.25b**	**84.583 a**	**83.583 a**	**5.7167a**	**5.9644a**	**5.9352a**	**12.499a**	**13.3798a**	**12.6113a**	**5.6148c**	**14.6925a**	**12.3335b**
MSD (P= 0.05)	**3.8458**			**0.3954**			**1.2447**			**0.6798**		
EMS	**62.8518**			**0.6644**			**6.5839**			**1.9640**		

Note: OAM= Over all mean, MSD= Minimum significant difference, EMS= Error mean square, Th= *Trichoderma harzianum*, Ta= *Trichoderma asperellum*, C= control and Tukey'sStudentized Range Test

Table 1b: Effect of bio-agents treatments on original seeds of maize through multi-pot tray method

	Fresh weight (g)			**Dry weight (g)**			**Seedling Length (cm)**			**Seedling Vigour index (Seedling length based)**			**Seedling Vigour index (Dry weight based)**		
Varieties	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**
V1	10.00 ±0.87	10.48 ±0.55	10.59 ±1.52	2.57 ±0.22	2.69 ±0.14	2.73 ±0.39	204.18 ±33.67	203.76 ±14.58	253.21 ±8.64	17160.29 ±3182.64	17961.45 ±2023.15	22608.62 ±3942.72	217.11 ±37.55	237.42 ±24.87	246.11 ±69.54
V2	9.85 ±1.30	10.44 ±1.00	11.32 ±0.88	2.46 ±0.33	2.61 ±0.25	2.83 ±0.22	218.75 ±71.92	297.50 ±20.62	245.00 ±17.32	14630.00 ±4804.26	21190.00 ±3244.75	18790.00 ±473.71	167.21 ±46.20	186.62 ±36.33	218.97 ±32.65
V3	8.50 ±0.71	9.88 ±0.85	10.00 ±0.82	2.27 ±0.19	2.64 ±0.23	2.67 ±0.22	180.00 ±74.94	227.50 ±17.08	242.50 ±11.90	12225.00 ±5094.82	17970.00 ±1940.82	19390.00 ±1678.53	155.13 ±26.44	209.46 ±36.63	214.67 ±34.90
V4	5.42 ±0.76	10.00 ±0.34	8.13 ±0.54	1.40 ±0.20	2.57 ±0.09	2.09 ±0.14	158.75 ±47.68	228.75 ±98.52	235.00 ±62.85	8465.00 ±3478.06	21970.00 ±9478.08	18560.00 ±5945.28	73.46 ±20.35	246.94 ±16.79	163.41 ±21.57
V5	9.21 ±0.68	9.21 ±0.94	9.09 ±0.74	2.37 ±0.17	2.37 ±0.24	2.34 ±0.19	204.18 ±33.67	195.62 ±35.18	203.73 ±15.81	16574.68 ±3129.73	15765.77 ±2546.64	16271.63 ±1473.52	192.40 ±28.58	193.10 ±38.82	187.85 ±30.54
V6	11.29 ±0.55	12.57 ±1.40	12.86 ±1.90	2.90 ±0.14	3.23 ±0.36	3.30 ±0.49	192.50 ±56.64	258.75 ±52.02	233.75 ±24.62	15230.00 ±4713.23	22425.00 ±2905.87	21060.00 ±4094.78	229.54 ±22.45	286.96 ±63.38	302.23 ±84.20
V7	13.50 ±1.00	15.67 ±1.15	15.33 ±1.44	3.47 ±0.26	4.03 ±0.29	3.94 ±0.37	191.25 ±80.45	266.25 ±64.21	226.25 ±74.09	15585.00 ±6955.54	25275.00 ±7646.67	21285.00 ±8490.91	282.19 ±41.92	380.02 ±55.79	364.94 ±66.23
V8	12.00 ±0.36	12.46 ±1.27	14.15 ±0.50	3.08 ±0.09	3.20 ±0.33	3.64 ±0.13	207.50 ±46.46	248.75 ±59.21	225.00 ±36.97	16140.00 ±3349.91	20240.00 ±5951.54	20710.00 ±3585.81	240.71 ±14.25	261.43 ±52.56	334.97 ±23.76
V9	10.30 ±0.49	12.12 ±1.40	12.12 ±1.57	2.65 ±0.13	3.12 ±0.36	3.12 ±0.40	218.75 ±25.94	255.00 ±44.35	242.00 ±17.30	14820.00 ±1177.79	20280.00 ±3508.77	19480.00 ±1423.24	180.37 ±17.30	251.71 ±57.56	252.33 ±64.41
V10	10.00 ±1.59	9.85 ±2.73	11.91 ±1.21	2.57 ±0.41	2.53 ±0.70	3.06 ±0.31	180.00 ±37.42	296.50 ±20.62	218.75 ±71.92	12230.00 ±3388.82	19750.00 ±4532.59	17910.00 ±7145.90	178.09 ±51.68	179.45 ±100.27	249.90 ±50.24
V11	12.62 ±0.35	13.39 ±1.62	13.23 ±1.07	3.24 ±0.09	3.44 ±0.42	3.40 ±0.28	186.25 ±53.60	231.25 ±92.77	196.25 ±65.75	15355.00 ±4761.24	20590.00 ±9276.40	16890.00 ±5990.16	266.02 ±14.97	302.55 ±69.26	293.85 ±45.67
V12	11.10 ±0.94	14.22 ±0.60	14.07 ±0.81	2.85 ±0.24	3.65 ±0.16	3.61 ±0.21	170.00 ±111.58	212.50 ±72.63	205.00 ±77.24	12145.00 ±8602.28	19315.00 ±6359.38	18420.00 ±6841.01	203.51 ±33.95	332.98 ±28.24	326.07 ±37.33
OAM	**10.315b**	**11.9002a**	**11.6898a**	**2.6520b**	**3.0602a**	**3.0604a**	**192.68b**	**243.59a**	**227.45a**	**14213.00b**	**20228.00a**	**19281.00a**	**198.809b**	**262.940a**	**255.718a**
MSD (P=0.05)	**0.5470**			**0.1406**			**26.935**			**2482.00**			**22.4230**		
EMS	**1.2713**			**0.0840**			**3082.967**			**26177918.00**			**2136.5740**		

Note: OAM= Over all mean, MSD= Minimum significant difference, EMS= Error mean square, Th= *Trichoderma harzianum*, Ta= *Trichoderma asperellum*, C= control and Tukey'sStudentized Range Test

Rolled paper towel method [20]

Data concerning to effect of bio-control agent on three seed categories are presented in **Tables 2a, and 2b**. Over all mean of all seed quality parameter of twelve maize seed sample that seed treatments with *Trichodermaharzianum* (Th) and *Trichoderma asperellum* (Ta) were significantly superior over control at level P=0.05. The six seed quality parameters *viz.* germination (GN), fresh weight (FW), dry weight (DW), seedling length (SL), seedling vigour index- based on seedling (SVSL) and seedling vigour index-based on dry weight (SVDW) were recorded by rolled paper towel method. In case of original seed treatment with *harzianum* (Th) and *harzianumasperellum* (Ta) excelled significant superior performance by contributing per cent germination 94.00±5.16 (V12) and 93.00±8.25 (V6), fresh weight (g) 12.25±1.26 (V1) and 13.00±0.82 (V3), dry weight (g) 3.68±0.38 (V1) and 3.90±0.24 (V3), seedling length (mm) 197.50±56.79 (V9) and 181.25±17.50 (V12), seedling vigour index-seedling length 18000.00±5275.15 (V9) and 16760.00±2475.05 (V6) and seedling vigour index- dry weight 341.70±17.73 (V4) and 355.80±46.99 (V6).

Twelve maize seed sample were treated with *Trichodermaharzianum* (Th) and *Trichoderma asperellum* (Ta) values of original seed i.e. per cent germination 86.000 and 85.083, fresh weight (g) 12.2292 and 11.7083, dry weight (g) 3.66875 and 3.5125, seedling length (mm) 183.542 and 172.292, seedling vigour index- seedling length 15810.000 and 14657.100 and seedling vigour index- dry weight 312.025 and 302.225. and significantly deference with control at level P=0.05.

Since there is no reports related to evaluation of seed treatment with bio-control agent through rolled paper towel method, hence results could not be compared.

Table 2a: Effect of bio-agents treatments on original seeds of maize through paper roll method

	Germination (%)			Fresh weight (g)			Dry weight (g)		
Varieties	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**
V1	84.00 ±7.30	87.00 ±6.00	86.00 ±9.52	12.25 ±1.26	12.25 ±1.26	12.75 ±1.26	3.68 ±0.38	3.68 ±0.38	3.83 ±0.38
V2	68.00 ±8.00	72.00 ±7.30	78.00 ±6.93	12.00 ±0.82	12.00 ±0.82	12.50 ±1.00	3.60 ±0.24	3.60 ±0.24	3.75 ±0.30
V3	69.00 ±5.03	78.00 ±5.16	81.00 ±6.83	12.25 ±1.26	12.00 ±0.82	13.00 ±0.82	3.68 ±0.38	3.60 ±0.24	3.90 ±0.24
V4	62.00 ±6.93	95.00 ±2.00	79.00 ±5.03	11.50 ±0.58	12.00 ±0.82	11.75 ±1.50	3.45 ±0.17	3.60 ±0.24	3.53 ±0.45
V5	82.00 ±5.16	82.00 ±7.66	82.00 ±5.16	11.75 ±0.50	11.75 ±0.50	12.75 ±1.26	3.53 ±0.15	3.53 ±0.15	3.83 ±0.38
V6	80.00 ±3.27	89.00 ±8.25	93.00 ±8.25	11.50 ±0.58	12.00 ±0.82	12.75 ±1.26	3.45 ±0.17	3.60 ±0.24	3.83 ±0.38
V7	80.00 ±3.27	89.00 ±8.25	86.00 ±5.16	11.00 ±0.82	11.75 ±0.96	12.00 ±0.82	3.30 ±0.24	3.53 ±0.29	3.60 ±0.24
V8	78.00 ±5.16	84.00 ±4.62	90.00 ±6.93	11.00 ±0.82	11.50 ±0.58	12.25 ±0.96	3.30 ±0.24	3.45 ±0.17	3.68 ±0.29
V9	70.00 ±2.31	91.00 ±3.83	83.00 ±6.83	9.00 ±0.82	11.75 ±0.50	10.75 ±1.26	2.70 ±0.24	3.53 ±0.15	3.23 ±0.38
V10	74.00 ±2.31	81.00 ±8.25	82.00 ±7.66	10.25 ±0.50	10.50 ±0.58	12.00 ±1.41	3.08 ±0.15	3.15 ±0.17	3.60 ±0.42
V11	82.00 ±2.31	90.00 ±5.16	90.00 ±2.31	11.25 ±0.96	11.50 ±0.58	12.25 ±0.96	3.38 ±0.29	3.45 ±0.17	3.68 ±0.29
V12	72.00 ±5.66		91.00 ±3.83	10.00 ±0.82	11.50 ±1.29	12.00 ±1.15	3.00 ±0.24	3.45 ±0.39	3.60 ±0.35
OAM	**75.083b**	**86.000a**	**85.083a**	**11.1458 c**	**12.2292 a**	**11.7083 b**	**3.34375 c**	**3.66875 a**	**3.5125b**
MSD (at 0.05)	**2.9046**			**0.4644**			**0.1393**		
EMS	**35.8518**			**0.9167**			**0.0825**		

Note: OAM= Over all mean, MSD= Minimum significant difference, EMS= Error mean square, Th= *Trichoderma harzianum*, Ta= *Trichoderma asperellum*, C= control and Tukey'sStudentized Range Test

Table 2b: Effect of bio-agents treatments on original seeds of maize through paper roll method

	Seedling Length (cm)			Seedling Vigour index (Seedling length based)			Seedling Vigour index (Dry weight based)		
Varieties	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**	**C**	**Th**	**Ta**
V1	107.50 ±18.93	177.50 ±61.31	168.75 ±17.50	8970.00 ±1401.09	15220.00 ±4596.75	14535.00 ±2401.74	307.80 ±30.63	243.75±1 62.75	327.90 ±39.96
V2	115.00 ±28.87	182.50 ±65.51	173.75 ±12.50	7800.00 ±1952.50	13030.00 ±4727.27	13500.00 ±749.40	244.80 ±32.79	258.90 ±28.54	292.20 ±32.50
V3	127.50 ±35.94	185.00 ±68.56	171.25 ±14.36	8770.00 ±2399.86	14330.00 ±5210.08	13800.00 ±295.75	253.80 ±33.16	280.20 ±16.05	314.70 ±10.48
V4	127.50 ±43.49	190.00 ±70.71	172.50 ±15.00	8100.00 ±3767.44	17980.00 ±6456.87	13660.00 ±1809.46	213.60 ±21.73	341.70 ±17.73	278.10 ±37.00
V5	130.00 ±40.82	180.00 ±63.25	166.25 ±16.01	10610.00 ±3145.77	14720.00 ±5381.65	13590.00 ±894.65	288.90 ±19.58	288.60 ±23.55	312.30 ±15.93
V6	126.25 ±37.28	182.50 ±59.09	180.00 ±18.26	10110.00 ±3042.35	16360.00 ±5996.09	16760.00 ±2475.05	275.70 ±9.91	321.30 ±47.62	355.80 ±46.99
V7	127.50 ±45.73	190.00 ±73.94	173.75 ±12.50	10200.00 ±3679.71	17180.00 ±7804.37	14925.00 ±1104.94	207.80 ±137.55	314.70 ±50.21	309.90 ±32.20
V8	130.00 ±42.43	192.50 ±56.79	161.25 ±10.31	9990.00 ±2609.60	16120.00 ±4653.09	14530.00 ±1704.39	258.30 ±35.70	289.80 ±21.59	330.30 ±31.59
V9	130.00 ±40.82	197.50 ±56.79	173.75 ±18.87	9130.00 ±3060.00	18000.00 ±5275.15	14355.00 ±1062.12	189.30 ±22.00	321.00 ±23.55	268.50 ±43.57
V10	128.75 ±42.11	180.00 ±67.33	168.75 ±10.31	9475.00 ±2869.81	14680.00 ±5941.40	13830.00 ±1491.80	227.40 ±8.85	255.90 ±37.35	295.20 ±44.81
V11	140.00 ±35.59	175.00 ±58.02	176.25 ±12.50	11510.00 ±3124.51	15910.00 ±5953.99	15875.00 ±1396.41	276.90 ±26.68	310.50 ±24.15	330.90 ±29.27
V12	118.75 ±35.21	170.00 ±54.77	181.25 ±17.50	8575.00 ±2846.44	16190.00 ±5981.96	16525.00 ±2109.68	216.60 ±29.55	325.20 ±49.20	328.50 ±44.45
OAM	**125.729b**	**183.542a**	**172.292a**	**9436.700b**	**15810.000a**	**14657.100a**	**251.45b**	**312.025a**	**302.225a**
MSD (at 0.05)	**21.083**			**1853.100**			**15.450**		
EMS	**1888.831**			**14592827.00**			**1014.433**		

Note: OAM= Over all mean, MSD= Minimum significant difference, EMS= Error mean square, Th= *Trichoderma harzianum*, Ta= *Trichoderma asperellum*, C= control and Tukey'sStudentized Range Test

Present study indicated that different species of *Trichoderma* extract treatments have affected seed growth parameters with a significant difference between control and treated seeds. The root and shoot lengths are the most important parameters for seed-borne mycoflora, beacause roots are in direct contacts with soil and absorb water from soil and supply it to the rest of the plant. For this reason root and shoot length provides an important clue plant response to seed mycoflora. The seedling length, fresh weight, dry weight, seedling vigour index- seedling length and seedling vigour index- dry weightshowed a highly significant difference among various verities in different treatments. Effects of interaction seed mycoflora and bio-control agents' treatment, germination, germination index, accumulative speed and coefficient of germination rate were showed a highly significant difference.

Akladious and Abbas [27] is also agreed and support that *Trichoderma harzianum*(T22) used as alternative to the chemicals to suppress the seed mycoflora and raise the yield of maize. Chlorophyll content increased when seed was coated with *Trichoderma harzianum*[28], also some species of *Trichoderma* have the ability to promote plant growth as well as improve plant defence level against biotic and/or abiotic stress [29,30,31]. Production of plant growth regulators, natural scarification, solublization of insoluble minor nutrients in soil, increased availability of micronutrients, increase in nutrient transfer from soil to root, Induced resistance to different pathogens (mostly other fungus) are the main *Trichoderma* mechanism of action in contact to plants [32,33,34,35].

Activation of each mechanism implies the production of specific compounds and metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen permeases[36,37]. These metabolites can be either overproduced or combined with appropriate bio-control strains in order to obtain new formulations for use in more efficient control of plant diseases and postharvest applications[36,38,39,40,41]. Our results show that the increased growth response of plants caused by application of some species of *Trichoderma* was more effective rather than other treatment, these results indicate that the method of *Trichoderma* introduction is also effective in the success of

Trichoderma in seedling growth improvement [42,43]. Improved plant growth might be due to increased solubility of insoluble plant nutrients by *Trichoderma* species [44]. Based on earlier report [45] and results presented here we conclude that plant growth may be improved by inoculation with *Trichoderma* spp. which leads to early emergence and also increased vigor of plants. Hence, our findings demonstrate that growth promoting properties of the *Trichoderma* strain improve the efficacy for commercial application.
They can also compete for infection sites on the root and can trigger plant defense reactions, inducing systemic resistance [46,47,48,49,50]. The competitive ability of a nonpathogenic strain partly determines its capacity to establish in soil and in the plant rhizosphere and is probably involved in its capability to colonize the root surface demonstrated that different strains have different capacities to colonize heat treated soil. In addition, saprophytic colonization of soil depends not only on the fungal strain but also on biotic and abiotic soil characteristics. Colonization of the root surface and root tissues probably depends not only on the fungal strain but also on the plant species and plant cultivar [27,30,51,52].

CONCLUSION

Nine parameters of seed quality was showed bio-agent like *Trichoderma harzianum* and *Trichoderma asperellum* treatment can effectively ensure high germination and seed vigour, which ultimately increase the productivity of crop. The potential of using *Trichoderma* isolates as enhancers of seed germination and plant growth and development could have important economic implications such as shortening the plant growth period and time, as well as improving plant vigor to overcome biotic and/or abiotic stresses, resulting in increased plant productivity and yields. On the basis of results, it is evident that some *Trichoderma* species are effective for growth promotion in maize. *Trichoderma* species are among the most-promising biocontrol fungi against many fungal plant pathogens. *T. harzianum* and *Trichoderma asperellum* have multiple mechanisms of action, including coparasitism via production of chitinases, ß-1-3 glucanases and ß-1-4 glucanases, antibiotics, competition, solubilization of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process.

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