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Biopriming Enhances Storage Stability, Plant Vigour, and Suppresses Wilt Disease in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

A study was carried out to evaluate the *in vitro* effect of three biopriming agents on the storage life of chickpea and suppression of wilt disease and found that *Trichoderma asperellum* was the best biopriming agent with a positive effect on biochemical parameters and plant health. Result on POX activity showed that with an increase of storage time, POX concentration was found to increase. But the rate of increase was comparatively low when seeds were bio-primed with *T. asperellum*. In case of alpha amylase activity, lowest concentration was recorded in the control but seeds bioprimed with *T. asperellum* showed highest concentration of alpha amylase activity. Highest protein content was recorded when *T. asperellum* was used as biopriming agent. On the other hand, highest TSS was recorded in control with lowest in bioprimed seeds. Out of the different priming time 6 hrs was found to be the best on biochemical parameters of chick pea at 9 months of storage. In pot culture, seeds primed with *T. asperellum* alone and combination with *B. bassiana* resulted in the highest plant growth parameters against *F.oxysporum* with higher seed production as compared to the seeds treated with chemical fungicides and other biocontrol agents. The present study showed that biopriming with *T.asperellum* and *B. bassiana* for 6 hrs shows a positive effect on biochemical parameters up to 9 months of storage of chickpea seeds with enhanced plant stand and growth parameters.

Keywords: Biochemical parameters, Chickpea, *F. oxysporum*, Bio-priming, Storage life

INTRODUCTION

Chickpea also known as Gram, Bengal gram, Garbanzo bean as well as Egyptian pea is an important crop among the pulses which is considered as king of pulses. It plays a specific role among the vegetarian population as it act as a source of protein. Per capita availability of pulses per day is only 47 g as against the minimum requirement of 104 g (WHO) (Vishwas *et al*.2017). India produces about 332.22 million tons of food grain (https://pib.gov.in) but losses of product remain static at 10% (Ali *et al*. 2015). The main reason for this is improper storage, and an average of 6% out of total 10% loss takes place during the storage of food grains. During storage, pests and diseases are the most important factors for losses of agricultural products. The microorganisms that attacks grains are very dangerous as they cannot be seen easily with the naked eye and their harmful influence spreads quickly and renders whole grains waste. In most cases, the storage fungi grows quickly at a temperature of about 20-40^0 C. The harmful storage microorganisms can cause human diseases such as liver diseases, gastrointestinal cancer, metabolic diseases, respiratory diseases, mental or psychological diseases and autoimmune diseases (Elshafei, 2021).

In India, more than 60% of the net area sown is under a rainfed system, it is quite appropriate to develop technologies for rainfed agriculture. Major problems of chickpea cultivation in the rainfed system are poor seed germination, seed dormancy, less plant vigour and disease and pest attacks. The major diseases are ascochyta blight, botrytis grey mould, *Fusarium* wilt, dry root rot, *Sclerotinia* stem rot (Bashir and Malik 1998) and *Calocobruchus bruchi* and pod borer are the major pests.

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceris* is a soil-borne pathogen that affects the chickpeas globally. *Fusarium* wilt causes maximum destruction to the crop, causing 100% loss in the highly infested field and under favourable environmental conditions (Jendoubi *et al*. 2017). The pathogen is known to cause two types of wilting symptoms. 'Early wilting' symptoms are flaccid leaves having dull-green discoloration, desiccation and crumpling of the entire plant within 25 days after

sowing while 'late wilting' symptoms are observed after 6–8weeks of sowing with drooped rachis, petioles and leaflets, first, in the upper part and then advances to throughout, leading to yellowing and necrosis of foliage (Younesi *et al.* 2021).

Seed priming is a method of pre-sowing treatment that partially hydrates seeds without allowing radical emergence. It is a controlled hydration process that involves in exposing seeds to lower water potentials that restrict germination, but permits pre-germination and causes physiological changes (Bradford 1986). Upon rehydration, primed seeds may exhibit a faster rate of germination, more uniform emergence, greater tolerance to environmental stresses and reduced dormancy in many species. Various priming methods like osmopriming, hydropriming, solid matrix priming, biopriming, nanopriming etc. are potentially used in seed technology. Biopriming is a new technique of seed treatment that integrates the biological and physiological aspects of seed growth and development and disease control. Nowadays, it is an alternative method of chemical fungicides for controlling the soil as well as seedborne pathogens.

The aim of the present study was to understand the effect of biocontrol agents (*Trichoderma asperellum*, *Metarhizium anisopliae* and *Beauveria bassiana*) on the biochemical parameters of chick pea seeds at different storage periods. Further study was carried out to study the performance of the bioprimed seeds in pot conditions on the reduction of wilt incidence and the effect on plant growth and yield parameters.

MATERIALS AND METHODS

The research work was carried out in the Nanotechnology Laboratory of the Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat during 2017-18 and 2018-2019. Chickpea seeds (Var. JG-16) were used to primed with three biopriming agents, *viz.*, *T.asperellum* (ITCC No. 8886.12), *M. anisopliae*(ITCC No. 8882.12) and *B.bassiana* (ITCC No. 8881.12),

Standardization of priming techniques

For standardization of biopriming, seeds were treated with three biopriming agents viz., *T. asperellum*, *M. anisopliae* and *B. bassiana* alone and in combination with each other for 1 hr, 2 hrs and 6 hrs and shade dried till moisture reached to 9 per cent. Seeds primed for 1 hr, 2 hrs and 6 hrs were then packed in HDPE semipermeable bags and stored for 9 months. Half of the seeds treated for 6 hrs were seeded in pot condition under shade net house.

Nine treatment combinations were tested with 5 replications and arranged in a Completely Randomized Design (CRD). The treatment combinations followed were,

T_1 = Control,

T_2 = Recommended (Malathion dust 5% @ 2.5g/kg+ Carbendazim @0.1%),

T_3 = Biopriming with *T. asperellum* (Ta)@ 5ml/litre (for 1 hr, 2 hrs, 6 hrs),

T_4 = Biopriming with *M. anisopliae* (Ma)@ 5ml/litre (for 1 hr, 2 hrs, 6 hrs),

T_5 = Biopriming with *B. bassiana* (Bb)@ 5ml/litre (for 1 hr, 2 hrs, 6 hrs),

T_6= Biopriming with *T. asperellum* + *M. anisopliae* @ 5ml/litre (for 1 hr, 2 hrs, 6 hrs),

T_7 = Biopriming with *M. anisopliae* + *B. bassiana*@ 5ml/litre (for 1 hr, 2 hrs, 6 hrs),

T_8 = Biopriming with *T. asperellum* + *B. bassiana*@ 5ml/litre (for 1 hr, 2 hrs, 6 hrs),

T_9 = Biopriming with *T. aperellum* + *M. anisopliae* + *B. bassiana*@ 5ml/litre (for 1 hr, 2 hrs, 6 hrs)

Effect of biopriming on biochemical parameters and storage life

Estimation of peroxidase activity

The peroxidase activity was estimated by using the methods of Putter (1974). Reagents used for doing peroxidase activity were Phosphate buffer 0.1 M (pH 7.0), Guaiacol solution 20 mM (0.24 ml guaiacol dissolved in 100 ml of water), Hydrogen peroxide solution (0.14 ml of 30 % H_2O_2 diluted in 100 ml of water).

For estimation of peroxidase, one gram (1g) of storage chickpea seeds were homogenized in 3 ml of phosphate buffer by grinding in a pre-cooled mortar and pestle and centrifuged the extract at 18,000 rpm at 5^0C for 15 minutes. The supernatant was used as an enzyme source by making 10 ml with phosphate buffer. 0.1 ml of enzyme extract was taken in a test tube and 3 ml of phosphate buffer was added, further 0.05 ml guaiacol solution and 0.03 ml of hydrogen peroxide solution were also added to the test tube.

The mixture was well shaken and placed in a spectrophotometer by putting the mixture in a cuvette. The time required for the mixture optical density to be increased by 0.1Δt at 436 nm was recorded and used in calculation. Since the extinction coefficient of guaiacol dehydrogenation product at 436 nm under the condition specified is 6.39 per micromole, the enzyme activity per litre of extract was calculated by using the formula,

Enzyme activity units/ litre = (3.18 x 0.1 x 1000/ 6.39 x 1 x Δt x 0.1)= 500/ Δt.

Estimation of alpha- amylase activity

For estimation of alpha-amylase activity, the method of Peter Bernfield (1955) was followed. The reagents used were 0.1M Sodium Acetate buffer (pH- 4.7), Acetic acid 0.2 M (1.5 ml glacial acetic acid was added in 100 ml distilled water), 1% starch solution (1 g starch was dissolved in 100 ml of 0.1 M Sodium acetate buffer), Dinitrosalicyclic (DNS) acid reagent (1g DNS, 0.2g Crystalline phenol and 0.05g sodium sulphite was dissolved in 100 ml of 1% NaOH), 40% Potassium Sodium Tartrate (PST) Standard Maltose solution (50 mg maltose was dissolved in 50 ml of distilled water).

For estimation of alpha –amylase activity, stored chickpea seeds were homogenized in 5 ml of ice cold 10 mM Calcium chloride solutions over night at 4^0C or 3 hours at room temperature and centrifuged the extract at 6,000 rpm at 4^0C for 10 minutes and the supernatant was made up to 10 ml with 10 mM calcium chloride. The supernatant was used as enzyme source. In a test tube, 1 ml of supernatant was taken and added with 1 ml starch solution, 2 ml DNS solution and 1 ml PST solution and mixed it properly by using vortex mixture and heat in a boiling water bath for 5 minutes. Dark orange red colour was developed and absorbance was measured at 560 nm against blank reagent. The alpha amylase activity was calculated by using standard curve prepared form maltose solution

Estimation of Total Soluble Sugar (TSS)

The total soluble sugar content was estimated by using the method of Yemm and Willis (1954). The reagents used for estimation of TSS were Ethanol (80% hot ethanol), Anthrone reagent (200 mg of anthrone reagents was dissolved in 100 ml of concentrated Sulphuric acid) and Standard glucose solution (100 mg of glucose was dissolved in 100 ml distilled water).

For estimation of TSS, stored chickpea seeds (20 mg) were homogenized in 5 ml of 80% hot ethanol and centrifuged at 4000 rpm for 20 minutes. The supernatant was collected and the residue was again centrifuged with 5ml of 80% hot ethanol at 4000 rpm for 20 minutes. Both the supernatants were mixed together. The known amount of ethanol extract was evaporated to dryness in a test tube on a water bath and cooled at room temperature. 1 ml of distilled water was added in a test tube and mixed thoroughly. To each test tube, 4 ml of anthrone reagent was added and mixed thoroughly and gently heated on water bath at 100^0 C for 10 minutes, cooled rapidly under running tap water and absorbance was measured at 630 nm against reagent blank. The amount of TSS was calculated using standard curve prepared from graded concentration of glucose. The concentration of TSS in the test tube were determined from the standard curve and expressed as per cent or mg/g dry weight.

Estimation of Total Protein (TP)

The total protein (TP) content was estimated by using the method of Lowry *et al.*(1951). The reagents used for estimation of total protein were Phosphate buffer (0.1 M, pH 7.6), Solution A: 2% Na_2CO_3 in 0.1 N NaOH (20 g of sodium carbonate and 4 g sodium hydroxoide was dissolved in distilled water of volume 1 litre), Solution B: 1% $CuSO_4.5H_2O$ solution (1 g of copper sulphate was dissolved in 100 of distilled water) and 2% potassium sodium tartarate was dissolved in 100 ml of distilled water. Both 1% $CuSO_4.5H_2O$ solution and 2% Potassium Sodium Tartarate was mixed in equal volume before use, Solution C: Carbonate- Cu++ solution (this solution was prepared by mixing 50 ml of Solution A and 1 ml of Solution B), Solution D:Folin- Ciocalteu Reagent (equal amount of water was added to the reagent before used, Standard Protein Solution: 25 mg of bovin serum albumin was dissolved in 100 ml of water.

For estimation of TP, one gram (1g) of chickpea seeds were homogenized in 5 ml of phosphate buffer and centrifuge at 4000 rpm for 20 minutes. The supernatant was collected and the residue was again centrifuged with 5ml of phosphate buffer at 4000 rpm for 20 minutes. Both the supernatant was mixed together. In 0.2 ml of phosphate extract, 5 ml of

Carbonate- Cu++ solution was added and mixed well by vortexing. After 10 minutes, 0.5 ml of Folin-Ciocalteu reagent was added and mixed well vortexing and absorbance was measured at 660 nm against blank reagent. The amount of TP was calculated by using standard curve prepared from Bovin serum albumin stock solution. The amount of protein in the test tube was calculated by standard curve and expressed as mg/g or 100 g sample.

Effect of biopriming against wilt of chick pea and plant growth and yield parameters:

The treatment combinations were further selected for pot experiment which was conducted in net house of Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, Assam. The treatment combinations were, T_1 = Uninoculated control (No seed treatment +No pathogen), T_2 = Inoculated control (*F. oxysporum*f.sp. *ciceri* @0.2%), T_3 = Recommended (Malathion dust 5% @2.5 g/kg + Carbendazim @0.1%) + *F. oxysporum*f.sp. *ciceri,* T_4 = Biopriming with *T. asperellum* (Ta)@5ml/litre + *F. oxysporum*f.sp. *ciceri*, T_5 = Biopriming with *M. anisopliae* (Ma) @5ml/litre + *F. oxysporum*f.sp. *ciceri*, T_6= Biopriming with *B. bassiana* (Bb)@5ml/litre + *F. oxysporum*f.sp. *ciceri,* T_7 = Biproming with *T. asperellum* + *M.anisopliae* @5ml/litre + *F. oxysporum*f.sp. *ciceri*, T_8= Biopriming with *M.anisopliae* + *B. bassiana* @5ml/litre + *F. oxysporum*f.sp. *ciceri*, T_9 = Biopriming with *T. asperellum* + *B. bassiana* @ 5ml/litre + *F. oxysporum*f.sp. *ciceri*, T_{10} = Biopriming with *T. asperellum* + *M. anisopliae* + *B. bassiana* @5ml/litre + *F. oxysporum*f.sp. *ciceri*

Statistical analysis

CRD was employed for the statistical analysis of the data. The data collected were subject to statistical analysis by Fisher's method of analysis of variance. Significance of variance among the data was analysed by calculating the "F" value and comparing it with the tabulated value of "F" at 5% level of probability.

The treatment means were compared among themselves by calculating critical difference (CD) as followed:

C.D. at 5%= S. Ed ×'t' 5% (at error d.f.)

The standard error of differences (S. Ed) was calculated by using the following formula:

S. Ed. =

$$\sqrt{\frac{2 \times \text{Error Mean Square}}{\text{Number of replication for each treatment}}}$$

Where, S. Ed. = Standard error of difference

't' 5% = "t" for error d.f. at 5% level of probability

The significance and non-significance of the treatments at 5% level of probability were calculated out by multiplying the S.Ed., with appropriate tabulated value for error degrees of freedom.

RESULTS

Effect of biopriming on peroxidase activity (POX) on chickpea at different storage period

The result shows that the peroxidase activity of storage chickpea seeds increases with the increase of storage period. The highest concentration of peroxidase activity was found in control while lowest in seeds bioprimed with *T. asperellum* in all priming time (1 hr, 2 hrs and 6 hrs). In case of 6 hrs priming seeds, though peroxidase activity increases with increase of storage period but in 9 months the increasing rate was lower as compared to other priming time (1 hr and 2 hrs) (**Table 1**).

Effect of biopriming on alpha amylase activity on chickpea at different storage period

As we know, Alpha amylase activity is an indicator of seed germination. In our present study, the alpha amylase activity was found to decrease with the increase of storage period. The highest alpha amylase activity was found in *T. asperellum-treated* seeds in all storage periods in all priming times (**Table 2**) followed by seeds primed with both *T. asperellum* and *B. bassiana.* The lowest alpha amylase concentration was found in control.

Effect of biopriming on total soluble sugar and total protein at different storage period

Highest protein content was recorded when *T. asperellum* was used as biopriming agent. Biopriming with *T. asperellum* + *B. bassiana* was found next to the biopriming *with T. asperellum* (**Table 3**).

Table 1: Effect of biopriming for 1 hr, 2 hrs and 6 hrs on Peroxidase activity of chickpea seeds at different storage periods

Treatments	Peroxidase activity											
	Time of biopriming											
	1 hr				2 hrs				6 hrs			
	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M
T_1	0.060	0.069	0.065	0.073	0.049	0.056	0.057	0.059	0.038	0.044	0.050	0.053
T_2	0.030	0.037	0.039	0.045	0.026	0.032	0.036	0.039	0.014	0.017	0.019	0.026
T_3	0.020	0.024	0.026	0.027	0.012	0.017	0.023	0.025	0.010	0.014	0.015	0.017
T_4	0.030	0.036	0.045	0.049	0.029	0.032	0.039	0.042	0.026	0.030	0.036	0.038
T_5	0.054	0.055	0.058	0.060	0.046	0.050	0.055	0.056	0.037	0.041	0.048	0.049
T_6	0.039	0.042	0.046	0.050	0.023	0.023	0.029	0.030	0.017	0.017	0.024	0.026
T_7	0.032	0.035	0.037	0.040	0.029	0.032	0.035	0.037	0.025	0.029	0.032	0.033
T_8	0.029	0.033	0.034	0.038	0.027	0.030	0.035	0.037	0.013	0.016	0.019	0.019
T_9	0.057	0.061	0.063	0.069	0.047	0.050	0.053	0.058	0.044	0.046	0.049	0.055
S.Ed (±)	0.019	0.028	0.039	0.026	0.039	0.024	0.013	0.042	0.074	0.040	0.006	0.046
CD (p=0.05)	0.039	0.040	0.078	0.052	0.078	0.048	0.027	0.084	0.148	0.080	0.012	0.092

*Data are mean of five replications

(T_1 : Control, T_2 : Recommended chemicals (Carbendazim @ 0.1% + Malathion dust @ 5%), T_3 : Biopriming with *T. asperellum* (T.a) @ 5 ml/litre, T_4 : Biopriming with *M. anisopliae* (M.a) @ 5 ml/ litre, T_5 : Biopriming with *B. bassiana* (B.b) @ 5ml/ litre, T_6 : Biopriming with *T.a* + *M.a* @ 5ml/ litre, T_7 : Biopriming with *M.a* + *B.b* @ 5ml/ litre, T_8 : Biopriming with *B.b* + *T.a* @ 5ml/ litre, T_9 : Biopriming with *T.a* + *M.a* + *B.b* @ 5ml/ litre)

Table 2: Effect of biopriming for 1 hr, 2 hrs and 6 hrs on Alpha amylase activity of chickpea seeds at different storage periods

Treatments	Alpha amylase activity											
	Time of biopriming											
	1 hr				2 hrs				6 hrs			
	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M
T_1	0.35	0.34	0.30	0.22	0.38	0.32	0.31	0.25	0.31	0.26	0.25	0.21
T_2	0.40	0.38	0.35	0.30	0.35	0.34	0.33	0.32	0.38	0.37	0.35	0.31
T_3	0.50	0.48	0.45	0.44	0.57	0.53	0.52	0.51	0.46	0.44	0.43	0.41
T_4	0.39	0.42	0.46	0.36	0.35	0.32	0.33	0.32	0.41	0.38	0.32	0.30
T_5	0.40	0.39	0.37	0.33	0.38	0.36	0.34	0.33	0.39	0.38	0.35	0.33
T_6	0.44	0.47	0.43	0.33	0.40	0.39	0.36	0.35	0.37	0.35	0.34	0.32
T_7	0.37	0.40	0.40	0.41	0.39	0.35	0.33	0.32	0.33	0.30	0.29	0.25
T_8	0.51	0.50	0.48	0.46	0.55	0.52	0.50	0.49	0.44	0.43	0.42	0.40
T_9	0.48	0.46	0.45	0.34	0.46	0.45	0.44	0.42	0.41	0.39	0.36	0.35
S.Ed (±)	0.02	0.05	0.07	0.08	0.02	0.04	0.08	0.09	0.01	0.06	0.07	0.12
CD (p=0.05)	0.05	0.10	0.16	0.16	0.04	0.08	0.16	0.18	0.02	0.12	0.14	0.24

*Data are mean of five replications, M: Months

(T_1 : Control, T_2 : Recommended chemicals (Carbendazim @ 0.1% + Malathion dust @ 5%), T_3 : Biopriming with *T. asperellum* (T.a) @ 5 ml/litre, T_4 : Biopriming with *M. anisopliae* (M.a) @ 5 ml/ litre, T_5 : Biopriming with *B. bassiana* (B.b) @ 5ml/ litre, T_6 : Biopriming with *T.a* + *M.a* @ 5ml/ litre, T_7 : Biopriming with *M.a* + *B.b* @ 5ml/ litre, T_8 : Biopriming with *B.b* + *T.a* @ 5ml/ litre, T_9 : Biopriming with *T.a* + *M.a* + *B.b* @ 5ml/ litre)

Table 3: Effect of biopriming for 1 hr, 2 hrs and 6 hrs on total protein (mg/g) of chickpea seeds at different storage periods

Treatments	Total protein (mg/g)											
	Time of biopriming											
	1 hr				2 hrs				6 hrs			
	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M
T_1	0.25	0.20	0.14	0.10	0.29	0.25	0.19	0.16	0.35	0.29	0.25	0.21
T_2	0.75	0.71	0.68	0.64	0.79	0.76	0.70	0.68	0.80	0.78	0.76	0.73
T_3	0.86	0.82	0.78	0.76	0.88	0.83	0.79	0.79	0.91	0.89	0.85	0.81
T_4	0.76	0.73	0.69	0.65	0.76	0.69	0.63	0.60	0.84	0.83	0.80	0.78
T_5	0.62	0.50	0.49	0.40	0.67	0.63	0.61	0.58	0.71	0.67	0.64	0.63
T_6	0.75	0.71	0.68	0.64	0.78	0.76	0.72	0.69	0.81	0.78	0.76	0.72
T_7	0.72	0.69	0.64	0.60	0.75	0.73	0.70	0.67	0.79	0.76	0.71	0.68
T_8	0.79	0.75	0.73	0.69	0.84	0.80	0.77	0.71	0.87	0.84	0.80	0.78
T_9	0.65	0.55	0.52	0.49	0.70	0.68	0.65	0.60	0.73	0.70	0.67	0.67
S.Ed (±)	0.09	0.01	0.09	0.01	0.10	0.01	0.10	0.01	0.04	0.04	0.10	0.14
CD (p=0.05)	0.18	0.02	0.18	0.02	0.21	0.02	0.21	0.02	0.08	0.08	0.2	0.28

*Data are mean of five replications, M: Months
(T_1 : Control, T_2 : Recommended chemicals (Carbendazim @ 0.1% + Malathion dust @ 5%), T_3 : Biopriming with *T. asperellum* (T.a) @ 5 ml/litre, T_4 : Biopriming with *M. anisopliae* (M.a) @ 5 ml/ litre, T_5 : Biopriming with *B. bassiana* (B.b) @ 5ml/ litre, T_6 : Biopriming with *T.a* + *M.a* @ 5ml/ litre, T_7 : Biopriming with *M.a* + *B.b* @ 5ml/ litre, T_8 : Biopriming with *B.b* + *T.a* @ 5ml/ litre, T_9 : Biopriming with *T.a* + *M.a* + *B.b* @ 5ml/ litre)

Lowest protein content was recorded in control. On the other hand, highest TSS was recorded in control with lowest in biopriming with *T. asperellum* (Table 4).

Effect of biopriming for 6 hrs and 9 months of storage on biochemical activity of chick pea seeds

The study on effect of bioprimed seeds in storage condition on biochemical activity showed that biopriming with *T.asperellum* @5ml/litre for 6 hrs has best in biochemical activities which increases the storage life of the chickpea seeds upto 9 months (Table 5). This was followed by seeds bioprimed with both the combination of *T. asperellum* and *B. bassiana.*

Effect of biopriming on physiological parameters of chickpea seeds in suppression of wilt disease

The effect of biopriming on physiological parameters of chickpea seeds are presented in Table 6. Results showed that all the treatments have a positive effect on all the physiological parameters with lowest seedling infection in seeds which were bioprimed for 6 hours. Highest per cent of seed germination (90%) was recorded when seed priming was done with *T. asperellum* alone and in combination with *B. bassiana.* These was followed by seed priming with *B. bassiana*, *T. asperellum* + *M. anisopliae*, *M. anisopliae* + *B. bassiana* and *T. asperellum* + *M. anisopliae* + *B. bassiana* without any significant difference among the treatment. Seeds priming with *M. anisopliae* showed 80.00% of seed germination which was better than the seeds without priming (60.76%). Lowest seed germination per cent (39.23%) was recorded in control, where seeds were neither primed with chemical nor any biocontrol agent (Table 6).The lowest PDI (6.77) wsa recorded in seeds priming with both *T. asperellum*and *B. bassiana.* This was followed by seed priming with *T. asperellum*(8.00%). Amongst the bioprimed seeds maximum PDI (11.99 %) was recorded when *B. bassiana*was alone was used as priming agents. Seeds primed with recommended chemical check showed PDI of 8.23%. On the other hand highest PDI of 90.00% was recorded in inoculated control.

Highest root length (16.36 cm) and shoot length (56.60) was recorded where seeds were treated with combination of *T. asperellum*and *B. bassiana*(Table 6). This was followed by seeds treated with *T.asperellum* alone. Amongst the primed seeds minimum shoot

length (43.60 cm) and root length (6.94 cm) was recorded in T_2 (Inoculated control). Seeds primed with recommended chemical check showed root and shoot length of 10.84 cm and 51.80 cm respectively (Table 6). The highest number of branches (5 nos.) were observed in seeds priming with both *T. asperellum* and *B. bassiana*. This was followed by seeds priming with *T.asperellum*, *B.bassiana* alone. Amongst the bioprimed seeds lowest number of branches was recorded in seeds primed with *M. anisopliae* (3 nos.) Seeds primed with recommended chemical showed 3 number of branches. Lowest number of branches (2 nos.) was recorded in inoculated control.Highest fresh weight of shoot (32.13 g) and root (1.63 g) was recorded when seeds were treated with combination of *T. asperellum*and *B. bassiana.* This was followed by seeds treated with *T. asperellum* alone. Amongst the primed seeds minimum fresh weight of shoot (12.16 g) and root (0.67 g) was recorded in T_2 (Inoculated control). Seeds bioprimed with recommended chemical check showed fresh weight of shoot and root of 17.92 g and 0.67 g respectively (Table 6). Similarly, highest dry weight of shoot (8.26 g) and root (0.65 g) was recorded when seeds were treated with combination of *T. asperellum*and *B. bassiana*. This was followed by seeds treated with *T. asperellum* alone. Amongst the primed seeds minimum dry weight of shoot (3.83 g) and root (0.29 g) was recorded in T_2 (Inoculated control). Seeds primed with recommended chemical check showed dry weight of shoot and root of 5.25 g and 0.31 g respectively. Data presented in Table 6 showed that highest seedling vigour index were recorded when seeds were primed with combination of both *T. asperellum* and *B .bassiana* (6625.80). This was followed by seeds treated with *T. asperellum* (5974.20). Amongst the bioprimed seeds minimum seedling vigour index was recorded in seeds treated with *M.anisopliae* (3353.39). Lowest seedling vigour index was showed in inoculated control (1982.68). Seeds primed with recommended chemical check showed the seedling vigour index of 3968.36.

Effect of biopriming on seed production of chickpea seeds in pot condition

Highest pods per plant was recorded when seeds were bioprimed with combination of both *T.asperellum* and *B. bassiana* (52 nos.). This was followed by seeds treated with *T.asperellum* (49 nos.). Amongst the primed seeds, the minimum pods per plant were recorded in the inoculated control (24 nos.). Seeds primed with recommended chemical check showed the pods per plant of 40 numbers. Data presented in Table 7 showed the highest seeds per pod in T9 and T4 where seeds were treated with *T. asperellum* alone and in combination with *B. bassiana* (2 nos.).

Table 4: Effect of biopriming for 1 hr, 2 hrs and 6 hrs on total soluble sugar of chickpea seeds at different storage periods

Treatments	Total soluble sugar											
	Time of biopriming											
	1 hr				2 hrs				6 hrs			
	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M	2 M	4 M	6 M	9 M
T_1	2.65	2.80	2.82	2.86	2.59	2.62	2.64	2.66	2.55	2.59	2.62	2.65
T_2	1.23	1.29	1.40	1.40	1.05	1.12	1.19	1.36	1.86	1.90	1.92	1.95
T_3	1.14	1.18	1.22	1.26	1.00	1.09	1.10	1.16	1.16	1.20	1.28	1.34
T_4	1.16	1.19	1.33	1.36	1.04	1.09	1.10	1.26	1.36	1.40	1.42	1.46
T_5	1.40	1.42	1.46	1.56	1.90	1.92	1.93	2.16	1.60	1.65	1.74	1.82
T_6	1.19	1.24	1.32	1.36	1.26	1.28	1.30	1.36	1.40	1.40	1.45	1.50
T_7	1.68	1.73	1.82	1.86	1.04	1.13	1.14	1.16	1.90	1.91	1.96	1.98
T_8	1.16	1.20	1.24	1.26	1.02	1.07	1.13	1.16	1.26	1.29	1.30	1.35
T_9	1.83	1.96	1.96	1.98	1.90	1.95	1.95	1.96	1.26	1.28	1.29	1.35
S.Ed (±)	0.120	0.12	0.05	0.08	0.09	0.08	0.06	0.06	0.14	0.10	0.04	0.04
CD (p=0.05)	0.24	0.24	0.10	0.16	0.18	0.16	0.12	0.12	0.28	0.2	0.08	0.08

*Data are mean of five replications, M: Months

(T_1 : Control, T_2 : Recommended chemicals (Carbendazim @ 0.1% + Malathion dust @ 5%), T_3 : Biopriming with *T. asperellum* (T.a) @ 5 ml/litre, T_4 : Biopriming with *M. anisopliae* (M.a) @ 5 ml/ litre, T_5 : Biopriming with *B. bassiana* (B.b) @ 5ml/ litre, T_6 : Biopriming with *T.a* + *M.a* @ 5ml/ litre, T_7 : Biopriming with *M.a* + *B.b* @ 5ml/ litre, T_8 : Biopriming with *B.b* + *T.a* @ 5ml/ litre, T_9 : Biopriming with *T.a* + *M.a* + *B.b* @ 5ml/ litre)

Table 5. Effect of biopriming for 6 hrs and 9 months of storage on growth parameters and biochemical activity of chick pea seeds (Summary table)

Treatments	Germin -ation (%)	Germina -tion index	Speed of germination	Seedling fresh weight (g)	Seedling dry weight (g)	Seedling vigour index	POX	Alpha amylase activity	Total soluble sugar (mg/g)	Total Protein (mg/g)
T1	50.09	82.00	39.30	0.55	0.18	350.12	0.053	0.21	2.65	0.21
T3	79.02	86.00	68.87	0.79	0.35	1028.84	0.017	0.41	1.34	0.81
T9	80.45	86.90	69.05	0.72	0.29	881.89	0.019	0.40	1.35	0.78
S.Ed (±)	1.01	1.62	1.56	0.02	0.03	8.96	0.046	0.12	0.04	0.14
CD(p=0.05)	2.22	2.65	3.25	0.04	0.06	16.92	0.092	0.24	0.08	0.28

Data are mean of five replications. T_1 : Control; T_3 : Biopriming with *T. asperellum* (T.a) @ 5 ml/ litre; T_9 : Biopriming with *T.a + M.a + B.b* @5ml/litre

Table 6. Effect of bio-priming on physiological parameters and seed production of chickpea in suppression of wilt disease

Treat-ments	Physiological parameters										Seed production			
	Time of biopriming (6 hours)													
	PDI	Seed germi natio n (%)	Shoot length (cm)	Root length (cm)	Branches (nos)	Fresh weight of shoot (gm)	Dry weight of shoot (gm)	Fresh weight of root (gm)	Dry weight of root (gm)	Seedling vigour index	Pods/ plant (nos)	Seed/ pod (nos)	Seed yield/ plant (gm)	1000 seed weight (gm)
T1	0.00	86.73	49.60	13.00	3.00	18.00	4.96	0.69	0.33	3186.71	37.00	2.00	1.55	121.80
T2	90.00	39.23	43.60	6.94	2.00	12.16	3.83	0.67	0.29	1982.68	24.00	1.00	1.05	53.80
T3	8.23	73.43	51.80	10.84	3.00	17.92	5.25	0.67	0.31	3968.36	40.00	1.00	1.47	180.40
T4	8.00	90.00	52.00	14.18	4.00	30.38	6.91	1.55	0.46	5974.20	49.00	2.00	1.64	236.80
T5	10.00	80.76	51.20	12.48	3.00	18.60	4.17	0.84	0.38	3353.39	44.00	1.00	1.38	171.00
T6	11.99	86.66	47.20	13.58	4.00	27.54	6.36	1.04	0.36	3843.80	24.00	2.00	1.48	211.80
T7	8.88	86.66	49.20	12.58	3.00	17.88	4.20	0.81	0.32	3906.34	46.00	1.00	1.45	149.80
T8	9.55	86.66	48.80	11.02	4.00	15.07	4.33	0.75	0.32	3782.41	33.00	1.00	1.37	181.00
T9	6.77	90.00	56.60	16.36	5.00	32.13	8.26	1.63	0.65	6625.80	52.00	2.00	2.02	239.80
T10	12.88	86.66	48.60	12.28	3.00	17.74	6.84	0.90	0.38	3849.43	33.00	1.00	1.05	192.40
SEd (±)	0.19	1.66	1.54	1.02	0.44	1.59	0.47	0.17	0.05	13.89	1.21	0.21	0.16	28.35
CD (p=0.05)	0.38	2.72	3.53	2.07	0.90	3.23	0.95	0.36	0.11	26.77	2.45	0.24	0.33	57.52

Data are mean of 5 replications.
(T_1: Uninoculated control (No seed treatment +No pathogen), T_2: Inoculated control (*F. oxysporum* f.sp. *ciceri* @0.2%), T_3: Recommended (Malathion dust 5% @2.5 g/kg + Carbendazim (@0.1%) + *F. oxysporum* f.sp. *ciceri*, T_4: Biopriming with *T. asperellum* (Ta)@5ml/litre + *F. oxysporum* f.sp. *ciceri*, T_5: Biopriming with *M. anisopliae* (Ma) @5ml/litre + *F. oxysporum* f.sp. *cicer,* T_6: Biopriming with *B. bassiana* (Bb)@5ml/litre + *F. oxysporum* f.sp. *ciceri*, T_7: Biproming with *T. asperellum* + *M.anisopliae* @5ml/litre + *F. oxysporum* f.sp. *ciceri*, T_8: Biopriming with *M.anisopliae* + *B. bassiana* @5ml/litre + *F. oxysporum* f.sp. *ciceri,* T_9: Biopriming with *T. asperellum* + *B. bassiana* @ 5ml/litre + *F. oxysporum* f.sp. *ciceri*, T_{10}: Biopriming with *T. asperellum* + *M. anisopliae* + *B. bassiana* @5ml/litre + *F. oxysporum* f.sp. *ciceri*)

Similarly, seed yield per plant (2.02 g) and 1000 seed weight (239.80 g) were recorded highest in seeds treated with both *T.asperellum* and *B. bassiana*. This was followed by seeds treated with *T.asperellum*. Lowest seed yield per plant (1.05 g) and 1000 seed weight (53.80 g) were recorded in inoculated control. Seeds primed with recommended chemical check showed the seed yield per plant and 1000 seed weight of 1.47 and 180.40 respectively.

DISCUSSION

Seed priming is a method of pre-sowing treatment that partially hydrates seeds without allowing radical emergence. It is a controlled hydration process that involves in exposing seeds to lower water potentials that restrict germination, but permits pre germination and occurs physiological changes (Bradford 1986). Biopriming is a new technique of seed treatment that integrates the biological and physiological aspects of seed growth and development and disease control. Now-a- days, it is an alternative method of chemical fungicides for controlling the soil as well as seed borne pathogens.

In the present study, peroxidase activity was found lowes tin *T. asperellum* primed seeds . This indicates that due to priming, firmness of seed increases resulting the more storage life. On the hands, alpha amylase activity though found decreases with increase of storage time in all the treatments, but the rate of decrease is lower in *T. asperellum* primed seeds. This indicates the low rate of decrease in the germination percentage of chickpea seeds in *T.*

asperellum primed seeds reduces compared to other treatments. With increase of storage the germination percentage along with other parameters were found decreases. This may be due to the reduction of concentration of protein that leads to increase in TSS concentration as observed in the present study. Earlier Dutta and Das (1999), Das *et al.* (2000), Dutta and Das (2008) reported that seed pelleting with *Trichoderma harzianum* can enhance the seed germination (%), reduced PDI and other plant growth parameter in rice, soybean, tomato and french bean, tomato etc. Ramegowda (1992) reported a decrease in the activity of enzymes *viz*., alpha-amylase, and peroxidase, coupled with progressive ageing of rice seeds. He further authenticated that alpha-amylase and peroxidase enzymes were more directly involved in the maintenance of better germination of differentially aged seeds. Failure of aged seeds to germinate might be due to peroxidation, mitochondrial disfunction and less ATP production changes in membrane lipids therefore could account for the increase in solute leakage (Zhou *et.al*. 2002). Out of the different bio-priming time 6 hrs was found to be the best which results in highest physiological parameter of chick pea at 9 months of storage with positive effect on biochemical parameters. In pot condition, the seeds which were primed with both the combination of *T. asperellum* and *B. bassaiana* showed the highest growth parameters as well as the seed production with reduced seedling infection as compared to inoculated control. It may be due to the increased water uptake and of *T. harzianum* and its translocation of nutrients as reported by Uddin *et al*.(2018). The seedling vigour index was also superior in bioprimed seeds. Mireshekari *et al*. (2012) reported that in barley maximum 1000 grain weight, grain yield were obtained by using nitrogen and phosphorus where fertilizers were treated with *Azospirillum +Azotobacter* as compared to control. Yadav *et al.* (2013), reported that the performance of rhizosphere microbial strains, viz., *Pseudomonas fluorescence*, *T.asperellum* and *Rhizopus* spp individually and in combination in bioprimed seeds of chickpea and rajma. The seeds that treated with biocontrol agents showed highest germination percentage and improve growth quality of plant as compared to control. Among all combination, the combination associated with *T.asperellum* showed better result than other combination and individual application. EL-Mohamedy *et al.* (2006) reported that cowpea seeds which were primed with *T.harzianum* reduced the root rot diseases by 64.0 % and 56.3 % at pre-emergence stage and by 68.0 %, 60.1% and 57.1 %, 64.0 % at post emergence stage after 40 and 60 days of sowing during 2004 and 2005 seasons respectively. Therefore, fresh pod, i.e., yield was increased as compared to Rizolex- T treatment during the same season. To conclude, the priming with *T.asperellum* and *B.bassiana* for 6 hrs can help to store the chick pea seeds up to nine months as we found positive result during study in biochemical parameters. The bioprimed seeds after storage for 9 months also showed positive results in suppressing the infection by *F. oxysporum* with increased plant growth and yield attributing parameters.

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