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BIO-STIMULANTS COATING FOR ENHANCED SEED VIGOUR AND LONGEVITY IN CHILLI (*CAPSICUM ANNUUM* L.)

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ABSTRACT

Bio-stimulants have gained recognition for boosting seed and seedling vigor by improving germination and field performance. They enhance stress tolerance, both abiotic and biotic. Seed coating with bio-stimulants promotes faster seedling growth, leading to healthier plants in nurseries. Present study highlights the effect of seed coating with bio-stimulants (C- dots, chitosan, humic acid, humic acid +binding agents, humic acid +fulvic acid, sea weed extract, Oxy Zn, *Trichoderma viride* (liquid formulation), *Trichoderma viride* (WP), signalling molecules, combination of fulvic acid with sea weed extract) on radicle emergence (%), field emergence and seed longevity in chilli. The results showed significant differences for radicle emergence and field emergence over a period of four months of storage. Immediately after treatment, chilli seed coated with T_5- humic acid +fulvic acid @ 2ml kg^{-1} showed significantly superior radicle and field emergence (90 ad 94, respectively) compared to control (70 and 80, respectively) and polymer (73 and 83, respectively). With the advancement in storage period, T_8-Oxy Zn also performed more or equally with T_5. After four months of storage period, T_8-OxyZn has improved 10% radicle emergence and field emergence compared to untreated control. The results concluded that seed treatment with humic acid +fulvic acid @ 2ml kg^{-1} and OxyZn @1 ml kg^{-1} performed better in maintaining seed vigour during storage and it could be recommended for seed treatment.

Key words : Biostimulants, Chilli, Seed vigour, Humic acid + fulvic acid, OxyZn.

Introduction

The global biological seed market size was valued at USD 1.28 billion in 2022 due to the adoption of biological seed coating by key players in the seed industry in view of the surging demand for chemical-free foods. It is expected to grow further at a compound annual growth rate (CAGR) of 12.4% from the year 2023 to 2030 (https://www.grandviewresearch.com).

In a recent EU Regulation, “plant biostimulant” has been defined as an EU fertilizing product which is applied to crop plants or rhizospheres with the aim of modulating plant physiological functions and of improving crop productivity, efficiency of nutrient use, quality of crop products and abiotic stresses tolerance (EU, 2019).

Microbial and non-microbial-based bio-stimulants significantly improve seedling & crop performance, in addition to disease suppression (Rouphael and Colla, 2020)

Plant bio-stimulants include several substances with bioactive properties: humic and fulvic acids, protein hydrolyzates, seaweed extracts, plant extracts and silicon, as well as plant growth promoting microorganisms: arbuscular mycorrhizal fungi (AMF), Trichoderma and plant growth promoting rhizobacteria. These can be applied to plants or soil to enhance seed germination, seedling vigour, crop growth, disease resistance, stress tolerance, nutrient availability and soil health. Unlike fertilizers, which directly provide essential nutrients to plants, bio-stimulants stimulate natural processes within plants and improve their tolerance to various environmental stresses

Materials and Methods

The present study was carried out at the Department of Seed Science and Technology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. Bio-stimulants were procured from Kaveri Microteck Private Limited, Secundrabad.

Seed treatment

Polymer slurry was prepared by mixing polymer (25 ml/kg) with distilled water (60 ml/kg). Different bio-stimulants at various concentrations were mixed with polymer and coated to chilli seeds (Table 1). Coated seeds were shade dried, packed in polythene pouches then stored under ambient conditions.

Table 1 : Treatment details of bio-stimulants used for chilli seed coating.

Treatment	Treatment details
T_1	C-dots @ (Fe- 0.25% @ 1.25 ml/kg+Mn-0.25%@ 1.25 ml/kg +B-0.25%@ 1.25 ml/kg +Zn-0.5%@ 2.5 ml/kg +Cu-0.25%@ 1.25 ml/kg) + Polymer @25 ml/kg + water@60 ml /kg seed
T_2	Chitosan @ 25ml/kg + Polymer @25 ml/kg + water@60 ml /kg seed
T_3	Humic acid @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg seed
T_4	Humic acid +Binding agents @ 2g/kg + Polymer @25 ml/kg + water@60 ml /kg
T_5	Humic acid + Fulvic acid @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_6	Fulvic acid (Amaze) @ 1ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_7	Sea weed extract @ 2ml/kg + Polymer @25 ml/ kg + water@60 ml /kg
T_8	OxyZn @ 1ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_9	*Trichoderma viride* (Biogaurd-L) @ 25ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_{10}	*Trichoderma viride* (Biogaurd-wp) @ 25g/kg + Polymer @25 ml/kg + water@60 ml /kg
T_{11}	Signalling molecules @1ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_{12}	Signalling molecules @2ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_{13}	Fulvic acid (Amaze) @ 1ml/kg + Sea weed extract @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg
T_{14}	$T_1+T_2+T_3$
T_{15}	Polymer @ 25 ml/kg + water@60 ml /kg
T_{16}	Untreated Control

To study the effect of bio-stimulants on seed quality enhancement and longevity, observations were recorded at bimonthly intervals for a period of four months on radicle emergence (%) and Field emergence (%)

The laboratory test for radicle emergence was conducted as per the ISTA rules (ISTA, 2022). Eight replicates of 25 seeds each were placed on the top of paper circles in each petri plate and kept in an incubator at an alternative temperature of 20^0C and 30^0C of 16 h at lower temperatures and 8 h at high temperatures. Radicle emergence was recorded at 6 hourly interval, 136 hours was finalized based on the final field emergence data and the number of seeds with 2 mm radicle was presented as percentage.

Field emergence test was performed by randomly selecting three hundred seeds from each treatment in three replicates. These seeds were sown at a depth of 1-2 cm in a well-prepared seed bed with adequate moisture. On the fourteenth day after sowing, the number of seedlings emerged above the soil surface were counted and recorded as a percentage.

The data recorded were analyzed statistically by adopting Completely Randomized Design (CRD), as described by Panse and Sukhatme (1985) and the standard error of difference was calculated at 5% probability level to compare the mean differences among the treatments. The data recorded as % was transformed to the respective angular (arc sin) values before subjecting them to statistical analysis.

Results and Discussion

With regard to the effect of bio-stimulants on chilli radicle emergence, the mean radicle emergence percentage was observed to be decreased gradually from zero months to four months after storage (80 to 70, respectively) with a drop down of 10 % in 4 months.

Immediately after coating, highest radicle emergence per cent was recorded by T_5-Humic acid+fulvic acid (90%), which was on par with T7-Sea weed extract (89%), T_8-OxyZn (88%) and T_9-*Trichoderma viride*-Liquid formulation (86). T_{16}-untreated control recorded least radicle emergence percentage (70%). Similar trend was observed two months after storage also. It might be due to phytohormones present in humic+fulvic acid which helps in germination. Immediately after coating with bio-stimulants all the treatments recorded an improvement in radicle emergence that ranges from 1 to 20% compared to untreated control (Fig. 1), more improvement in radicle emergence was recorded by T_5 (20%) followed by T_7 (19%) and T_8 (18%). When compared to polymer, more increase in radicle emergence was observed in T_5

followed by T_7 and T_8 (17, 16 and 15, respectively). Two months after storage, all the treatments showed improvement in percentage radicle emergence over untreated control and polymer (Fig. 2).

Four months after storage, T_8 (84%) recorded highest radicle emergence which was on par with T_5 (83%) and T_7 (82%), whereas, 60% was recorded in T_{16}- untreated control. Increase in radicle emergence per cent might be due to Zn, which helps production of growth hormone auxin leads to early emergence of radicle. But lowest radicle emergence per cent was recorded by T_{10}- *Trichoderma viride*-Wettable powder formulation (50). This might be due to high dosage of solid biological material used for coating, in addition to that carrier material used might have obstructed the moisture penetration into the seed which might have acted as a physical barrier for germination intiation.

Four months after storage (Fig. 3), all the treatments showed similar trend in change in per cent radicle emergence over polymer and untreated control. T_8 recorded highest improvement in radicle emergence over control and polymer (24 and 22, respectively) followed by T_5 (23 and 21, respectively).

Pandya and Mehta (2021) reported that the seed treatment with green marine bio-stimulant *Ulva lactuca* on germination and seed vigour in *Vigna radiata*. Similarly in the present study seed treatment with bio-stimulants performed best in seed vigour test *i.e.*, Radicle emergence test.

Qiu *et al*. (2020) reported that seeds of red clover and perennial ryegrass coated with different combinations of bio-stimulants (soy flour, diatomaceous earth, micronized vermicompost, and concentrated vermicompost extract) increased seedling vigour in both the crops compared to untreated control.

Effect of seed coating with bio-stimulants on Field Emergence (%) and seed longevity in chilli

Field emergence was observed to be decreased gradually with the advancement of seed storage period. The mean field emergence per cent was decreased from zero to four months after storage (85 to 72%, respectively) as presented in the Table 3.

Immediately after coating, T_5- Humic acid+fulvic acid and T_7-Sea weed extract (94 and 94, respectively) recorded highest field emergence percentage, which was on par with T_8-OxyZn (90%) compared to T_{16}-untreated control (80%). But lowest field emergence was recorded with T_{12}-signaling molecule at high dosage 2ml/kg seed (79). Compared to untreated control and polymer (Fig. 4), T_5 (14 and 11, respectively) and T_7 (14 and 11,

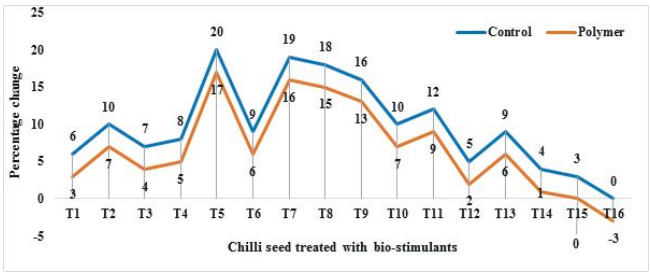

Fig. 1 : Percentage change in radicle emergence of bio-stimulants coated chilli seed over untreated control and polymer at 0MAS.

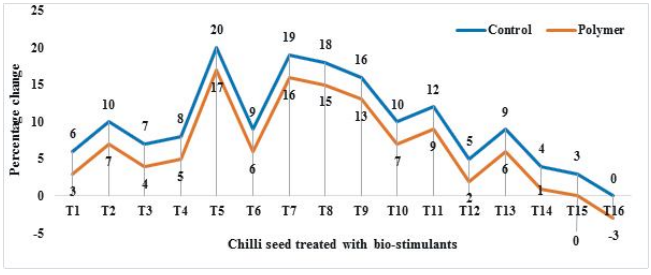

Fig. 2 : Percentage change in radicle emergence of bio-stimulants coated chilli seed over untreated control and polymer at 2MAS.

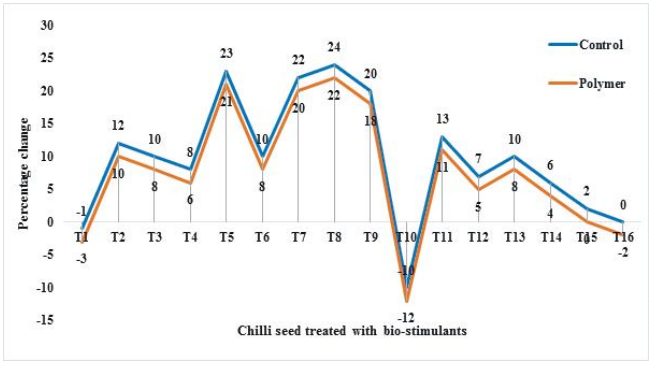

Fig. 3 : Percentage change in radicle emergence of bio-stimulants coated chilli seed over untreated control and polymer at 4MAS.

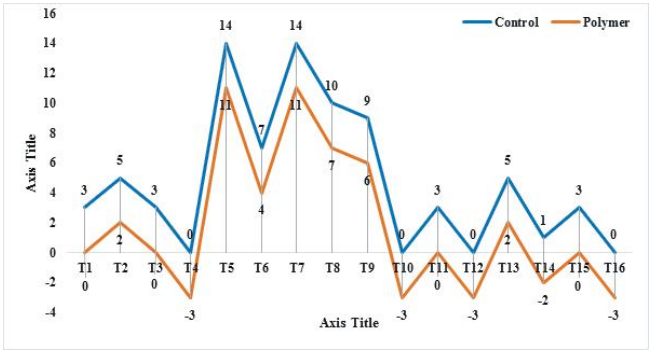

Fig. 4 : Percentage change in field emergence of bio-stimulants coated chilli seed over untreated control and polymer at 0MAS.

respectively) showed more increase in field emergence per cent at zero months of storage.

Two months after storage, T_5- Humic acid+fulvic

Table 2 : Effect of seed coating with bio-stimulants on Radicle emergence (%) and seed longevity at 136 hours in chilli.

Treatment	Treatment details	Radicle Emergence (%)		
		0MAT	2MAT	4MAT
T_1	C-dots @ (Fe- 0.25% @ 1.25 ml/kg+Mn-0.25%@ 1.25 ml/kg + B-0.25%@ 1.25 ml/kg +Zn-0.5%@ 2.5 ml/kg +Cu-0.25%@1.25 ml/kg) + Polymer @25 ml/kg + water@60 ml /kg seed	76	72	59
T_2	Chitosan @ 25ml/kg + Polymer @25 ml/kg + water@60 ml /kg seed	80	76	72
T_3	Humic acid @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg seed	77	72	70
T_4	Humic acid +Binding agents @ 2g/kg + Polymer @25 ml/kg + water@60 ml /kg	78	74	68
T_5	Humic acid + Fulvic acid @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg	90	86	83
T_6	Fulvic acid (Amaze) @ 1ml/kg+Polymer @25 ml/kg+ water@60 ml/kg	79	75	70
T_7	Sea weed extract @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg	89	85	82
T_8	OxyZn @ 1ml/kg + Polymer @25 ml/kg + water@60 ml /kg	88	85	84
T_9	*Trichoderma viride* (Biogaurd-L) @ 25ml/kg + Polymer @25 ml/kg + water@60 ml /kg	86	83	80
T_{10}	*Trichoderma viride* (Biogaurd-wp) @ 25g/kg + Polymer @25 ml/kg + water@60 ml /kg	80	78	50
T_{11}	Signalling molecules @1ml/kg+Polymer @25 ml/kg+water@60 ml/kg	82	77	73
T_{12}	Signalling molecules @2ml/kg+Polymer @25 ml/kg+water@60 ml /kg	75	71	67
T_{13}	Fulvic acid (Amaze) @ 1ml/kg + Sea weed extract @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg	79	74	70
T_{14}	$T_1+T_2+T_3$	74	70	66
T_{15}	Polymer @ 25 ml/kg + water@60 ml /kg	73	69	62
T_{16}	Untreated Control	70	66	60
	Mean	80	76	70
	C.D.(0.05)	**3.742**	**4.14**	**3.87**
	SE(m)	**1.23**	**1.37**	**1.28**
	SE(d)	**1.75**	**1.94**	**1.81**
	C.V.	**2.20**	**2.55**	**2.60**

acid showed highest field emergence percentage 89 which was on par with T_7-Sea weed extract (86%) and T_8-OxyZn (86%) against T_{16}-untreated control (76). Compared to T_{16}-untreated control and T_{15}-polymer, chilli seed coated with T_5 recorded more improvement in field emergence with 13 and 12%, respectively (Fig. 5).

After four months of storage, T_8-OxyZn (82) recorded highest field emergence percentage which was on par with T_5- Humic acid+fulvic acid, T_7- Sea weed extract and T_9- *Trichoderma viride*-Liquid formulation (80, 79 and 79, respectively) while T_{16}-untreated control showed 70%. But chilli seed coated with T10-*Trichoderma viride*-Wettable powder formulation recorded significantly lowest field emergence per cent (46). More improvement in field emergence was recorded by T_8 (12 and 4 %, respectively) followed by T_5 (10 and 2%, respectively) compared to untreated control and polymer but more reduction was recorded by T_{10} (-24 and -32, respectively) as presented in Fig. 6.

Chemical composition of bio-stimulants might have enhanced the biological mechanism in the growing seedlings that might have helped in the cell repair, elongation and division which might have led to higher emergence.

These results are in accordance with findings of Oliveira *et al.* (2023), who reported that seed treatment with bio-stimulants increased seedling emergence

Table 3 : Effect of seed coating with bio-stimulants on Field Emergence (%) and seed longevity in chilli.

Treatment	Treatment details	Field emergence (%)		
		0MAT	2MAT	4MAT
T_1	C-dots @ (Fe- 0.25% @ 1.25 ml/kg+Mn-0.25%@ 1.25 ml/kg + B-0.25%@ 1.25 ml/kg +Zn-0.5%@ 2.5 ml/kg +Cu-0.25%@1.25 ml/kg) + Polymer @25 ml/kg + water@60 ml /kg seed	83	78	58
T_2	Chitosan @ 25ml/kg + Polymer @25 ml/kg + water@60 ml /kg seed	85	82	72
T_3	Humic acid @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg seed	83	81	70
T_4	Humic acid +Binding agents @ 2g/kg + Polymer @25 ml/kg + water@60 ml /kg	80	78	73
T_5	Humic acid + Fulvic acid @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg	94	89	80
T_6	Fulvic acid (Amaze) @ 1ml/kg+Polymer @25 ml/kg+water@60 ml/kg	87	84	73
T_7	Sea weed extract @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg	94	86	79
T_8	OxyZn @ 1ml/kg + Polymer @25 ml/kg + water@60 ml /kg	90	86	82
T_9	*Trichoderma viride* (Biogaurd-L) @ 25ml/kg + Polymer @25 ml/kg + water@60 ml /kg	89	83	79
T_{10}	*Trichoderma viride* (Biogaurd-wp) @ 25g/kg + Polymer @25 ml/kg + water@60 ml /kg	80	76	46
T_{11}	Signalling molecules @1ml/kg+Polymer @25 ml/kg+water@60 ml/kg	83	82	77
T_{12}	Signalling molecules @2ml/kg +Polymer @25 ml/kg+water@60 ml/kg	80	75	71
T_{13}	Fulvic acid (Amaze) @ 1ml/kg + Sea weed extract @ 2ml/kg + Polymer @25 ml/kg + water@60 ml /kg	85	81	78
T_{14}	$T_1+T_2+T_3$	81	78	72
T_{15}	Polymer @ 25 ml/kg + water@60 ml /kg	83	77	74
T_{16}	Untreated Control	80	76	70
	Mean	**85**	**81**	**72**
	C.D. (0.05)	**3.96**	**4.17**	**4.00**
	SE(m)	**1.31**	**1.38**	**1.32**
	SE(d)	**1.85**	**1.95**	**1.87**
	C.V.	**2.18**	**2.42**	**2.59**

Note: MAS- Months After Storage.

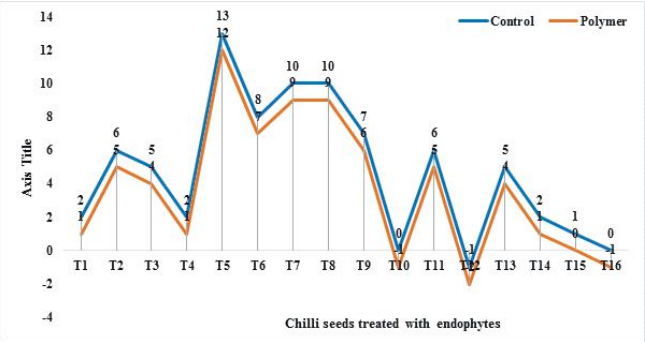

Fig. 5 : Percentage change in field emergence of bio-stimulants coated chilli seed over untreated control and polymer at 2MAS.

percentage and speed of emergence in the field compared to untreated control.

Similar to current study Asaduzzaman *et al.* (2010) reported that microbial biostimulant *Trichoderma* species have potential to enhance the germination in chili seeds both in field and lab conditions which can be useful to enhance the germination percentage of chili seeds besides reducing loses due to delayed germination.

Makhaye *et al.* (2021) stated that bio-stimulants, such as seaweed extracts, humic substances and microbial inoculants, can enhance germination processes by improving metabolic efficiency and positively influence

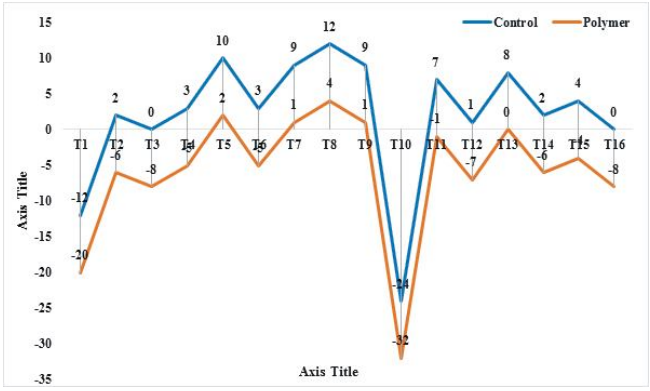

Fig. 6 : Percentage change in field emergence of bio-stimulants coated chilli seed over untreated control and polymer at 4MAS.

hormonal metabolism, leading to improved germination rates.

Conclusion

Seed treatments with different bio-stimulants increased radicle emergence, field emergence and seed longevity in chilli. It was revealed that immediately after coating combination of humic acid + fulvic acid +polymer showed highest radicle and field emergence per cent, with the advancement of storage period OxyZn + polymer also perfomed well. It was concluded that treatment with bio-stimulants along with polymer improved seed vigour.

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