



New Plant Growth Regulator ‘Verva’ and its Application on Wheat

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Abstract. Plant growth regulators (PGRs) exert physiological effects on plant growth, development and defence, and are thus crucial in agricultural production. Verva, a novel PGR, exhibits multiple functions, high efficiency and environmental sustainability. This study elucidated the effects of Verva on plant growth, development and defence and determined efficient and precise techniques for its field application. Verva was extracted from fir trees and used in wheat seed germination tests and hydroponics experiments. Moreover, the antagonistic activities of Verva on five pathogenic fungi (*Fusarium oxysporum*, *Fusarium verticillioides*, *Botryosphaeria dothidea*, *Botrytis cinerea* and *Fusarium proliferatum*) were evaluated using the plate test. Field experiments were conducted to evaluate the effect of the combined use of Verva and *Trichoderma* Tv-1511-based bio-organic fertiliser on wheat growth and yield. Verva improved wheat seed germination, seedling growth and biomass production, eliciting an initial increase followed by a subsequent decrease with increasing concentration, with the optimal concentration at 0.5‰ (V/V). Verva also exhibited potent antifungal properties against all tested fungi at inhibitory rates of 41.34%–64.27% at 0.5 ‰ (V/V) concentration. Tv-1511 exhibited a 1.35-fold increase in growth compared with the control group, after treatment with 0.5 ‰ (V/V) Verva for 24 h. Furthermore, the combined application of Verva and *Trichoderma*-based bio-organic fertiliser significantly enhanced wheat growth and yield. The yield of Dongying and Rizhao wheat after combination treatment with Verva and *Trichoderma*-based bio-organic fertiliser increased by 22.28% and 21.99%, respectively, which are significantly higher than that of conventional fertiliser ($P < 0.01$).

Keywords: Plant growth regulator; Verva; wheat; growth promotion and productivity increase.

1 Introduction

Plant growth regulators (PGRs) are exogenous plant hormones that are directly extracted or artificially synthesised from plants and exhibit the same or similar physiological and biological effects as natural plant hormones [1,2]. PGRs are important in regulating the growth and development of plants [3], increasing crop yields [4], enhancing resilience [5] and improving plant quality [6]. The use of PGRs to regulate plant growth and development is now one of the most important applications in agricultural production [7].

PGRs derived from conifer forests have recently become widely used. Among these is Verva, a biological preparation extracted from fir produced by the Institute of Chemistry of the Komi Research Center of the Urals Branch of the Russian Academy of Sciences. This preparation is an alkaline solution consisting of phenolics, essential oils, terpenes and diterpene fatty acid salts. The main active ingredient of this formulation is a natural terpenoid acid sodium salt exerting bioactivities that are important for plant growth and disease resistance [8]. Verva demonstrates robust efficacy at low concentrations and exhibits micro-efficiency. Furthermore, Verva is eco-friendly and non-toxic; thus it has a low environmental impact. Research on Verva is currently focused in the north (Komi Republic and Kirov region), centre (Saratov, Moscow and Bashkir regions) and south (Kuban) of Russia; studies on Verva in China are scarce. The present study investigated the inhibitory effect of Verva, a novel plant growth regulator, on pathogenic fungi, as well as on seed germination and growth in wheat. Furthermore, the effect of combining Verva with wood mould bio-organic fertiliser on wheat growth and yield was explored.

Results of this study would facilitate the wider application of Verva in China and help reduce the use of chemical pesticides and fertilisers as part of the 13th Five-Year Plan.

2 Materials and Methods

2.1 Test Materials

Reagents for testing: Verva. Verva is preserved by the Institute of Biology of the Qilu University of Technology (Shandong Academy of Sciences).

Strains: *Trichoderma viride* Tv-1511, preserved at the Institute of Biology, Qilu University of Technology (Shandong Academy of Sciences).

Test crop: Wheat (Jimai 22).

Test medium: Potato dextrose agar (PDA) solid medium.

Wood mould bio-fertiliser for testing: Golden Star (Rizhao) Agricultural Technology Co. provided this. The solid fermentation of Tv-1511 was added at 5% of the dry weight of bio-organic fertiliser and with a spore count of $2 \times 10^8/\text{g}$.

2.2 Test Methods

2.2.1 Wheat Seed Germination Experiment. Wheat seeds that were full and of uniform size were selected, surface-sterilised with 2% sodium hypochlorite for 3 minutes, rinsed four to five times with sterile water and air-dried in an ultra-clean bench until the seed surface was free of water. A double layer of filter paper was placed in a Petri dish with a diameter of 11 cm. Then, 10 mL of different concentrations of Verva [0.005%, 0.05%, 0.5% and 5% (V/V)] were added to the Petri dish, with sterile water used as the control. Subsequently, 20 seeds were meticulously dispersed within each dish, with four replicates being allocated for each treatment. The germination rate and number of lateral roots of the seeds was counted, and the radicle length and germ length were determined.

2.2.2 Effect of Verva Against Pathogenic Fungi. Five plant pathogenic fungi, namely, *Fusarium oxysporum*, *F. moniliforme*, *B. dothidea*, *B. cinerea* and *F. proliferatum*, were selected for testing. Pieces (5 mm in diameter) were taken from the edge of the fungal colonies, and Verva was inoculated on PDA plates at concentrations of 0.005%, 0.05%, 0.5% and 5% (V/V). A plate without Verva was used as the control. The plates were placed in a constant-temperature incubator, and the diameter of the growth rings of the pathogenic fungi was measured daily by noting the morphology and size of the growth rings. The fungal inhibition rate was calculated as follows:

Inhibition rate = [(diameter of control pathogen colonies – diameter of treated pathogen colonies) / diameter of control pathogen colonies] × 100%.

2.2.3 Effect of Verva on T. Viride Growth. Pieces (5 mm diameter) were taken from the edge of the green Tv-1511 colonies using a hole punch and inoculated on PDA plates with a Verva concentration of 0.5% (V/V). Plates without Verva treatment were used as control. The plates were placed in a constant-temperature incubator, and the diameters of the growth rings of Tv-1511 were measured every 12 h. The morphology and size of the growth rings were scanned, and the inhibition rate was calculated.

2.2.4 Wheat Seedling Growth Promotion Trials. Full and uniform wheat seeds were selected for germination and nursery. After cultivation for a certain period of time, seedlings with uniform growth were selected and transferred to a hydroponic unit for treatment. A hydroponic solution prepared using ½ Hoagland nutrient solution was used as the control, whereas the treatment group consisted of various concentrations of Verva [0.005%, 0.05%, 0.5% and 5% (V/V)]. Four replicates were established for each treatment. Plant height, root length, fresh weight and dry weight were measured after 7 days of treatment.

2.2.5 Field Trials. The experiments were conducted at Jinxing (Rizhao) Agricultural Science and Technology Co. Ltd. and the Yellow River Delta Agricultural Hi-Tech

Industrial Demonstration Zone in Shandong Province. CK (no fertiliser) and CF (conventional fertiliser) served as controls.

T1: CF + 0.5‰ Verva foliar spray.

T2: 70% CF + 30% Tv-1511 bio-organic fertiliser.

T3: 70% CF + 30% bio-organic fertiliser + 0.5‰ Verva foliar spray.

Three replicates were established per treatment. All treatments were fertilised uniformly with the required nutrients and routinely managed according to local fertilisation practices.

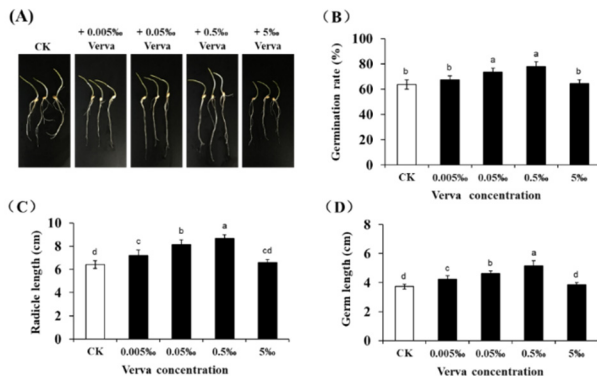
2.3 Data Handling

All experimental data were tabulated using Excel 2010, and SPSS 20.0 software was used for statistical and significance analyses. The results are expressed as mean $\pm$ standard deviation.

3 Results and Analysis

3.1 Effect of Verva on Wheat Seed Germination

The different concentrations of Verva treatments varied in the levels of effect on wheat seed germination. In the 0.005‰–0.5‰ concentration range, Verva effectively promoted germination rate, radicle length and germ length of wheat seeds and the effects are enhanced with increasing Verva concentration. However, seed germination was inhibited when the Verva concentration was increased to 5‰. The germination percentage, radicle length and germ length of wheat in the 0.5‰ Verva treatment group increased by 22.35%, 35.15% and 38.61%, respectively, a significant difference ($P < 0.05$) compared with that in the control group (Fig. 1). Thus, Verva promoted wheat seed germination, with the most significant effect observed at 0.5‰ concentration.



Note: CK is not added with Verva. Different letters above the columns indicate significant differences between treatments at the level of $P < 0.05$.

Fig. 1. Effect of Verva on wheat seed germination.

3.2 Antagonistic Effect of Verva on Pathogenic Fungi

Verva showed a significant antagonistic effect against the five plant pathogens, which increased with concentration (Fig. 2, Table 1). At 0.05‰ concentration, the inhibition rates against *F. oxysporum*, *F. moniliforme*, *B. dothidea*, *B. cinerea* and *F. proliferatum* were 33.07%, 42.30%, 34.82%, 33.82% and 51.51%, respectively. At 0.5‰ concentration, the inhibition rates were 41.35%, 45.70%, 34.82%, 41.62% and 64.27%, respectively. Moreover, the antagonistic effects of Verva at 0.5‰ and 5‰ concentrations against *F. oxysporum*, *B. dothidea* and *B. cinerea* did not differ significantly ($P > 0.05$). Thus, Verva effectively inhibited the growth of pathogenic fungi and achieved better fungal inhibition at 0.5‰ concentration.

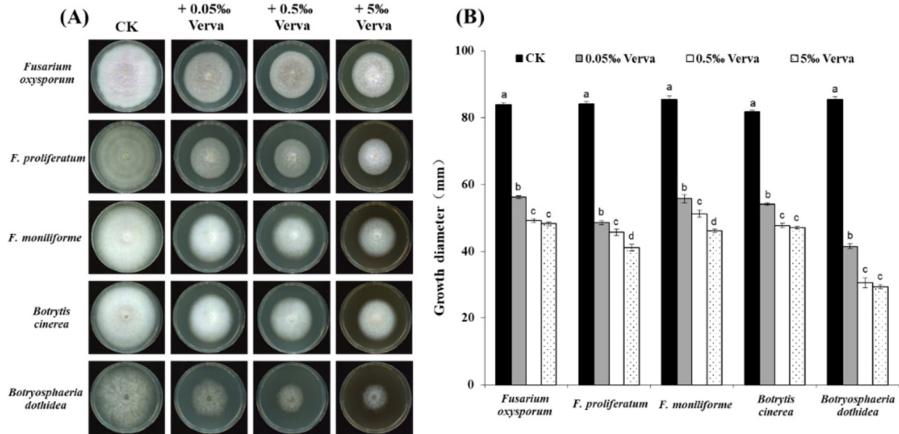
3.3 Effect of Verva on the Growth of Tv-1511

Tv-1511 was inoculated onto PDA plates with 0.5‰ Verva and evaluated for growth performance. As demonstrated in Fig. 3, Verva significantly enhanced the growth of Tv-1511. At 24, 36, 48 and 60 h of incubation, the growth diameter of Verva-treated Tv-1511 was 1.34, 1.15, 1.15 and 1.14 times higher than that of the control group, respectively, all of which were significant ($P < 0.05$ or $P < 0.01$). Therefore, Verva can significantly promote the growth of *T. viride*. As the fungicides and bio-organic fertilisers prepared with *T. viride* more significantly enhanced growth and increased yield, they can be effectively applied in combination with Verva. Tv-1511 was inoculated onto PDA plates with 0.5‰ Verva and evaluated for growth performance. As demonstrated in Fig. 3, Verva significantly enhanced the growth of Tv-1511. At 24, 36, 48 and 60 h of incubation, the growth diameter of Verva-treated Tv-1511 was 1.34, 1.15, 1.15 and 1.14 times higher than that of the control group, respectively, all of which were significant ($P < 0.05$ or $P < 0.01$). Therefore, Verva can significantly promote the growth of *T. viride*. As the fungicides and bio-organic fertilisers prepared with *T. viride* more significantly enhanced growth and increased yield, they can be effectively applied in combination with Verva.

Table 1. Verva's inhibition rate on five fungi

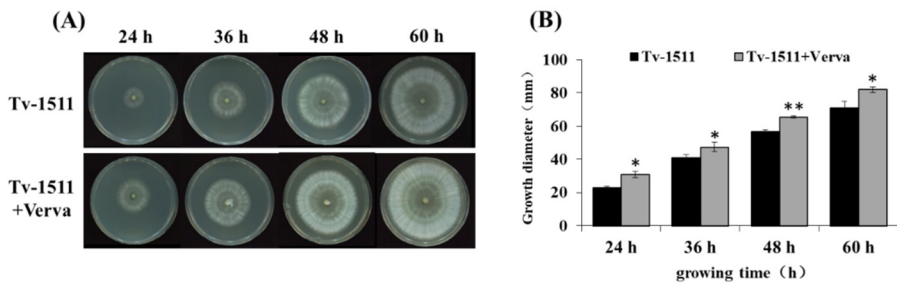
	0.05‰ Verva	0.5‰ Verva	5‰ Verva
<i>F. oxysporum</i>	33.07±1.00b	41.35±0.90a	42.56±0.10a
<i>F. Moniliforme</i>	34.82±1.40c	40.02±1.30b	46.02±0.70a
<i>Botryosphaeria dothidea</i>	51.51±0.90b	64.27±1.60a	65.63±0.70a
<i>Botrytis cinerea</i>	33.82±0.40b	41.63±0.80a	42.38±0.30a
<i>F. proliferatum</i>	42.30±0.70c	45.71±1.10b	51.14±1.10a

Note: The data in the figure are the mean ± se (n=4), and different letters in the same column indicate the significance of differences at the level of $P < 0.05$.



Note: CK is not added with Verva. Different letters above the columns indicate significant differences between treatments at the level of $P < 0.05$.

Fig. 2. Antagonism of Verva against five pathogenic fungi.

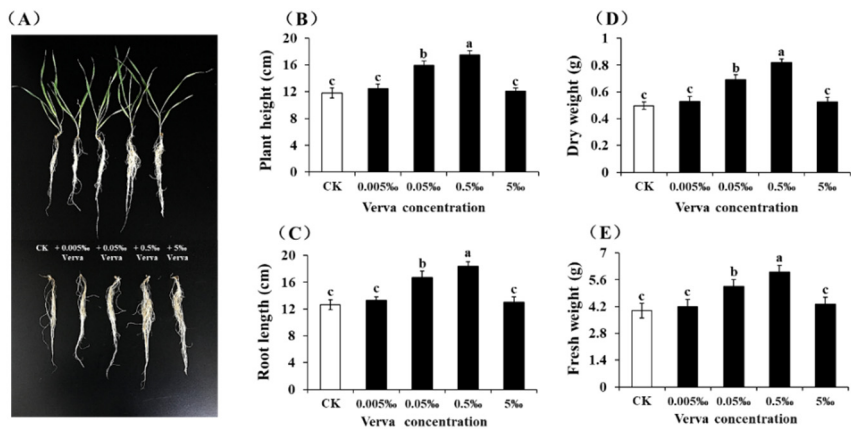


Note: the * and ** on the column indicate that the difference between the treatment group and the control group is significant ($P < 0.05$) or extremely significant ($P < 0.01$),

Fig. 3. Effect of Verva on *Trichoderma viride* growth.

3.4 Effect of Verva on Wheat Growth

Verva enhanced growth (Fig. 4), with greater growth rates at higher concentrations, especially 0.5% (Fig. 4A). The effects of Verva concentrations on wheat seedlings were evaluated using plant height, root length and dry and fresh weights. Results revealed consistent trends with the germination-related indexes, peaking at 0.5%. Specifically, treatment with 0.5% Verva concentration increased plant height, root length, dry weight and fresh weight by 47.69%, 45.73%, 64.82% and 50.22%, respectively, compared with the control group. Thus, Verva significantly promoted the growth of wheat seedlings and increased biomass, with the most significant growth-promoting effect at 0.5% concentration.

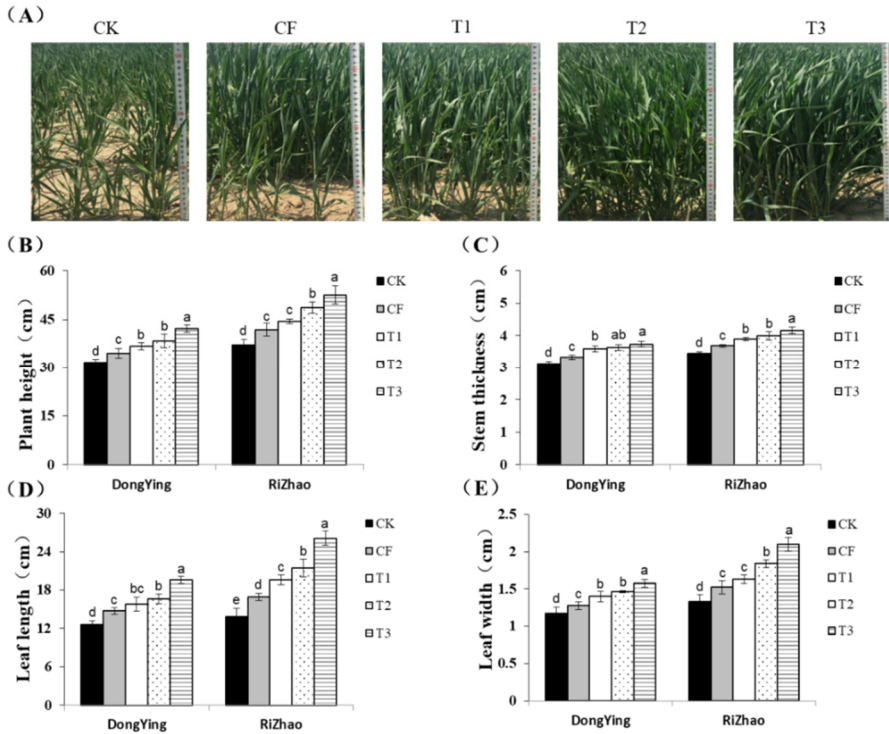


Note: CK is not added with Verva. Different letters above the columns indicate significant differences between treatments at the level of $P < 0.05$.

Fig. 4. Effect of Verva on the growth and biomass of wheat seedlings.

3.5 Effect of Combining Verva with Tv-1511 Bio-Organic Fertiliser on Wheat Growth and Yield

Experiments were carried out in Dongying and Rizhao to evaluate the effect of the combination of Verva and Tv-1511 bio-organic fertiliser on field wheat growth (Fig. 5). Compared with CF, all fertilisation treatments significantly promoted the growth of wheat in Dongying and Rizhao. However, the most effective combination was the treatment with 30% Tv-1511 bio-organic fertiliser + foliar spraying with 0.5‰ Verva (T3). In Dongying, the plant height, stem thickness, leaf length and leaf width of T3-treated wheat were 1.22, 1.21, 1.33 and 1.24 times higher than those of CF, respectively. In Rizhao, the corresponding indexes of the T3-treated group were 1.26, 1.13, 1.54 and 1.38 times higher than those of CF, respectively. Therefore, Verva combined with Tv-1511 bio-organic fertiliser significantly enhanced wheat growth and resistance. To evaluate the effect of fertilisers on the yield of field wheat, the spike number, spike length and thousand-grain weight were determined. Results (Fig. 6) were consistent with the initial growth trend of field wheat, with the T3 treatment group demonstrating the best yield enhancement. In Dongying wheat, the spike length, number of grains in the spike, and thousand-grain weight in the T3-treated group all significantly increased ($P < 0.05$) by 1.16, 1.08 and 1.32 times, respectively, compared with those in CF-treated wheat. In Rizhao wheat, the corresponding indexes in the T3 treatment group increased by 1.18, 1.16 and 1.23 times compared with those in the CF-treated group.



Note: CK: no fertiliser; CF: conventional fertiliser; T1: conventional fertiliser + Verva; T2: 30% Tv-1511 bio-organic fertiliser; T3: 30% Tv-1511 bio-organic fertiliser + Verva.

Fig. 5. Effects of different fertilisation treatments on the growth of Dongying and Rizhao wheat.

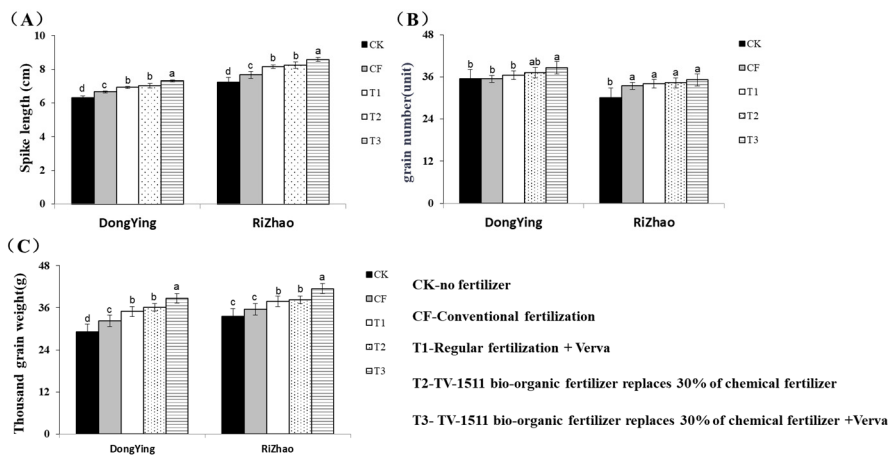


Fig. 6. Effects of different fertilisation treatments on the yield of Dongying and Rizhao wheat.

The mu yields of the Dongying and Rizhao wheat were determined. As demonstrated in Table 2, the yields of Dongying wheat under different treatments were 5.0% higher in T1, 8.4% higher in T2 and 13.6% higher in T3. The yield in the T1 group was 8.4% higher than that in the CF group, whereas the yields in the T2 and T3 groups were 13.68% and 22.28% higher, respectively ($P < 0.01$). In Rizhao wheat, the yields increased in the T1, T2 and T3 groups by 9.6%, 12.61% and 21.99%, respectively, compared with the CF group ($P < 0.01$). Bio-organic fertilisation with Tv-1511 and optimal Verva concentration significantly increased the wheat yield, surpassing the effectiveness of either Tv-1511 bio-organic fertiliser or Verva applied alone.

Table 2. yield differences of wheat under different fertilization treatments

	Dong Ying			Ri Zhao		
	Productivity (kg/hm ²)	Significant differ- ences		Productivity (kg/hm ²)	Significant differ- ences	
		$P<0.05$	$P<0.01$		$P<0.05$	$P<0.01$
CK	4258.95±14.53	d	D	4846.65±32.61	d	D
CF	5270.4±4.03	c	C	5795.4±13.39	c	C
T1	5713.65±10.74	b	B	6355.65±16.44	b	B
T2	5991.9±16.29	b	B	6526.8±7.09	b	B
T3	6444.9±27.06	a	A	7070.1±20.16	a	A

Note: The uppercase and lowercase letters in the table represent significant differences at $P < 0.01$ and $P < 0.05$.

4 Discussion and Conclusions

PGRs can significantly improve seed germination, promote seedling growth and increase crop yield [9]. PGRs applied endogenously can regulate plant root development and growth, break apical dominance, promote lateral shoot growth, alter plant flowering time and counteract external stresses [10]. However, the effectiveness of PGRs depends on the concentration. Although they promote growth at optimal levels, this effect diminishes with increasing concentration and may even be inhibitory. Many PGRs, such as indoleacetic acid and gibberellin, exhibit physiological effects that are promoted at lower concentrations and inhibited at higher concentrations [11]. Srinivasulu et al. [12] found that low concentrations of GA4+7+6BA (a combination of gibberellin and benzyladenine) promoted the germination of lime seeds, whereas the stimulating effect diminished with increasing concentration, even showing toxicity and absence of seed germination at 1,000 mg/L. In the present study, Verva at 0.5‰ concentration optimally enhanced wheat seed germination, seedling growth and yield, with no significant differences with those at higher concentrations. Therefore, Verva as a plant growth regulator can exert a strong promotional effect but needs to be limited to lower concentrations.

PGRs not only significantly promotes plant growth and improves stress resistance but also effectively controls some plant diseases and improve plant disease resistance

[13]; Baskaran et al. [14] found that thiamethoxam and naphthalene acetic acid exerted some bacteriostatic activity against *Enterococcus faecalis*, *Mycobacterium luteum* and *Staphylococcus aureus* in begonia. Similarly, Verva inhibited the pathogenic fungi *F. oxysporum*, *F. moniliforme*, *B. dothidea*, *B. cinerea* and *F. proliferatum*, an effect that may be attributed to the essential oils or phenols in Verva [15]. Furthermore, Verva has a broad-spectrum antagonistic effect on phytopathogenic bacteria, although it does not affect the growth of *T. viride*. Thus, PGRs can be applied in combination with a microbial fungicide. Plant growth regulator–microbe interactions have important applications in agriculture. By prudently utilising these interactions, plant yield and disease resistance can be substantially enhanced [16,17]. Giron et al. [18] found that PGRs can enhance plant growth and development by mediating plant interactions with parasitic and symbiotic organisms. Furthermore, Spence et al. [19] found that abscisic acid (ABA), another plant growth regulator, affects the pathogenicity of various bacterial and fungal pathogens and promotes resistance during plant–pathogen interactions. The present study demonstrated that, Verva, a key regulator of plant–microbe interactions, can majorly enhance the growth, yield and resistance of wheat to diseases and adversities in combination with lignocellulosic-type bio-organic fertilisers, which has potential for commercialisation and other applications.

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