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Integration of salicylic acid, vermicompost and bioagent for effective management of chickpea wilt disease

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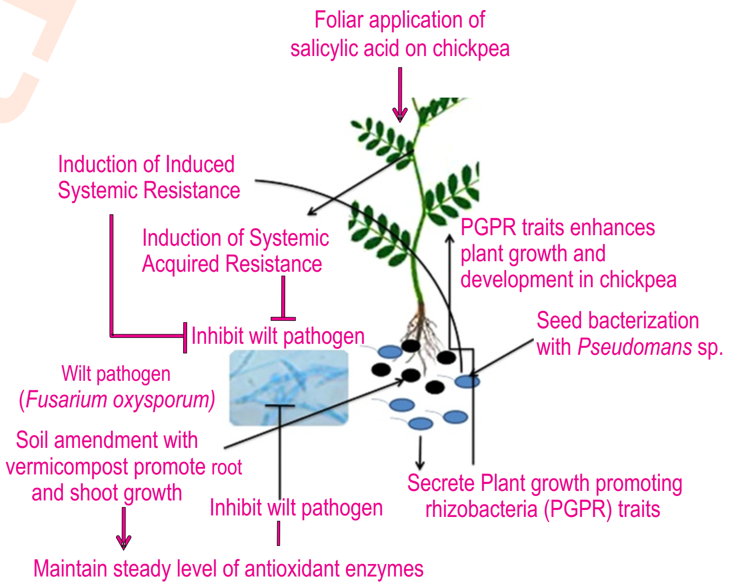
Abstract

Aim: The aim of the present study was to evaluate the ability of integration of salicylic acid, vermicompost and bioagent for effective management of chickpea wilt disease.

Methodology: The effectiveness of salicylic acid and $ZnSO_4$ unaided and in combination with Plant growth-promoting rhizobacteria (PGPR) and vermicompost were evaluated against *Fusarium* wilt of chickpea under natural condition. Three sets of experiment with nine treatments were conducted in earthen pots in completely randomized design. Ten seeds of wilt susceptible chickpea variety JG 62 were sown. Twenty-days-old plants were sprayed with salicylic acid (Set I), $ZnSO_4$ (Set II) and distilled H_2O (Set III). After 24 hr of foliar spray, the whole set of treatment was inoculated with *Fusarium oxysporum* f. sp. *ciceri* inoculums, except uninoculated control. The number of wilted seedlings in each pot for each treatment were recorded at 10, 20 and 30 days post-inoculation (dpi) and compared with uninoculated pots.

Results: The combined effect of vermicompost amendment @15% and pre-inoculation treatment of salicylic acid showed 0.00, 6.67 and 6.67% wilt incidence whereas treatments having $ZnSO_4$ as pre-inoculation foliar spray resulted in 0.00, 13.33 and 13.33% wilt incidence at 10, 20 and 30 dpi, respectively. Further, the combined treatment of 15% vermicompost along with seed bacterization and pre-inoculation foliar spray of salicylic acid showed complete protection against *F. oxysporum* f. sp. *ciceri.* The beneficial effect of vermicompost and PGPR isolate on root and shoot length, and fresh and dry weight of chickpea plants were also observed.

Interpretation: High potential for integrating vermicompost, bioagent and foliar application of salicylic acid to surrogate chemical fungicides for eco-friendly and sustainable management of wilt disease in chickpea.



Key words: Chickpea wilt, Pseudomonad, Salicylic acid, Vermicompost

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Introduction

Chickpea (*Cicer arietinum* L.) is the second important food legume crop after common bean (*Phaseolous vulgaris* L.) with a total cultivated area of 17.81 million hectares having production of 17.19 tonnes world wide. India shares approximately 67 % of world area and 66% of global production of chickpea (FAO STAT, 2018). Owing to its high nutritional value, chickpea has occupied foremost place among the legume crop production in India. Chickpea production and productivity is vulnerable due to occurrence of various biotic stresses (Sharma *et al.*, 2019). Among various biotic stresses, Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is the prime constraints, which reduced yield ranging from 10–100% depending on varietal susceptibility and prevailing climatic situations (Jendoubi *et al.,* 2017).

Different fungicides have been used for controlling wilt disease. Since this disease is soil borne, chemical control method is neither much effective nor eco-friendly (Sharma *et al.*, 2017). A plethora of reports indicate the management practices for controlling this devastating disease (Merkuz and Getachew, 2012; Mohamed *et al.,* 2016). Mostly management of this devastating disease was carried out using change in sowing date, use of beneficial microorganisms, organic amendments, other chemicals and fungicides (Mengist *et al.,* 2018; Kaur *et al.,* 2018). However, effective managament of this disease is limited by following different management practices separately, which argue the necessity to integrate different management practices. Further, today's agriculture practices focused on cost effective and sustainable management of diseases. Triggering induced resistance in plant has emerged as effective management strategies against several diseases (Choudhary *et al.,* 2007). Salicylic acid, a well-known inducer of systemic acquired resistance, has been used for controlling wilt pathogens in different crops (Dihazi *et al.,* 2011). Reports indicate the use of PGPR (plant growth promoting rhizobacteria), which induces induced systemic resistance for controlling wilt disease (Avinash and Ravishankar, 2017; Kim *et al.,* 2020). Evidence for induction of systemic acquired resistance in plants provides broad term resistance against several diseases but it also incurred fitness cost (Reynolds *et al.,* 2019). Although several studies have reported the potential use of PGPR for plant growth promoting activities along with sustainable management of diseases, there has been limited use of PGPRs due to variability and inconsistency of results (Gouda *et al.,* 2018). Therefore, a collective approach which combines the potential of systemic acquired resistance and induced systemic resistance can enhance the defense mechanism in plant besides their individual effect (Choudhary *et al.,* 2007). Several reports advocate soil amendment with vermicompost, which helps to build a pool of available nutrient near root zone to support the plant growth and beneficial microbes (Sahni *et al.,* 2008; Piya *et al.,* 2018). In a recent study, Sahni and Prasad (2020) have shown that PGPR strain in combination with vermicompost amendment enhanced disease resistance against collar rot by producing low but uniform level of defense enzymes in chickpea (Sahni and Prasad, 2020). In this study, a combined application of vermicompost, salicylic acid, and seed bacterization were investigated for effective wilt disease management and enhanced plant growth and dry matter accumulation in chickpea, with the goal of developing environmentally friendly agriculture.

Materials and Methods

Vermicompost amendment: Sandy loam soils (pH 7.2) were sampled from a depth of 5–15 cm from the research farm of Tirhut College of Agriculture, Dholi, Muzaffarpur, Bihar, India. Gravel and stubble debris were removed manually from the collected soil and sterilized using autoclave. Vermicompost @ 10, 15 and 20% v/v were carefully mixed with sterilized soils. Soils without mixing vermicompost were used as a control. Equal amount of either soil or vermicompost-amended soil were filled in the earthen pots (inner dimension 20 x 27 sq. cm).

Seed bacterization: Seeds of a wilt susceptible chickpea variety JG 62 were used for the experiment. Pseudomonad isolate (PGPR-WS) was used for seed bacterization as described by Weller and Cook (1983). Seeds coated with only slurry of carboxymethyl cellulose served as control.

Chemical treatment: Foliar applications of salicylic acid (40 ppm) (Merck, Germany) and $ZnSO_4$ *(50 ppm)* (Merck, Germany) were used in this study.

Fungal inoculums: Inoculum of *F. oxysporum* f. sp. *ciceri* was prepared by soaking chickpea seeds (JG 62) in 2 % sucrose solution overnight, surface dried, filled into conical flasks (@250g per flask) and autoclaved at 15 psi for 20 min. The cooled sterilized flasks were inoculated with *F. oxysporum* f. sp. *ciceri* by adding 8 mm agar plugs using sterile cork borer and incubating the inoculated flasks at 25ºC for 7 days. At the time of inoculation, each flasks containing inoculum was mixed in 2 kg of potting mixture containing sterilized soil and vermicompost, which was used to fill the earthen pots (inner dimension 20 x 27 sq. cm) to develop a artificial wilt sick field.

Treatment details

Seed bacterization and vermicompost amendments: A pot experiment was carried out in earthen pots (inner dimension 20 x 27 sq. cm) under natural conditions to evaluate the consequence of seed bacterization and different doses of vermicompost amendments in soil alone and in combination on root and shoot growth, fresh and dry weights of chickpea plants. T1 = Control (soil), T2 = vermicompost amendment @ 10% v/v, T3 = vermicompost amendment @ 15% v/v, T4 = vermicompost amendment @ 20% v/v, T5 = seed bacterization with PGPR-WS, T6 = T2 + T5, T7 = T3 + T5, and T8 = T4 + T5 were the eight treatments evaluated. Five seeds of JG 62 treated with 1 % CMC were sown in pots with treatments T_1 - T_4. Similarly, five seeds of JG 62 treated with PGPR-WS were sown in pots containing treatments T_5 - T_8. Treatment T_1, seeds treated only with 1% CMC served as control. Each treatment comprised of 5 pots and each pot was considered as single replication. The pots were arranged in completely randomized design and grown under natural condition. After 50 days of sowing, one plant from each of the five pots comprising each treatment was carefully uprooted and analyzed for root and shoot length (cm), and fresh weight (g). For dry weight estimation, uprooted plant were washed carefully and dried at 65 °C in hot air oven till it attend a constant weight.

Treatment of Salicylic acid, $ZnSO_4$, seed bacterization and vermicompost: In order to evaluate the effectiveness of salicylic acid (40 ppm) and $ZnSO_4$ (50 ppm) unaided and in combination with PGPR and vermicompost, three sets (I, II and III) of experiment were conducted in earthen pots (inner dimension 20 x 27 sq. cm) containing same amount of soil under natural condition. Each set of experiment consisted of 9 treatments. The treatments were as follows: T_1 = Control (uninoculated), T_2 = Control (inoculated with *F. oxysporum*), T_3 = Vermicompost amendment @ 10%, T_4 = Vermicompost amendment @ 15%, T_5= Vermicompost amendment @ 20 %, T_6 = Seed bacterization with PGPR-WS, $T_7 = T_3 + T_6$, $T_8 = T_4 + T_6$ and $T_9 = T_5 + T_6$. Ten seeds of JG 62 treated with 1% CMC were sown in pots with treatments T_1-T_5, whereas ten seeds of JG 62 treated with PGPR-WS were sown in pots with treatments T_6 - T_9. Treatment T_1 and T_2 without vermicompost amendment and seed treatment with PGPR-WS served as controls, in which seeds were treated only with 1% CMC. Each treatment consisted of three pots and each pot served as single replication. The pots were arranged in completely randomized design.

After 20 days of sowing, germinated chickpea seedlings were sprayed with salicylic acid (Set I), $ZnSO_4$ (Set II) and distilled H_2O (Set III). After 24 hr, the whole set of treatment, except for uninoculated control (T_1) was inoculated with *F. oxysporum* inoculum as mentioned above and applied to the top one third area of the pots in each treatment. The number of wilted seedlings in each pot for each treatment was recorded at 10, 20 and 30 days post inoculation (dpi) and compared with uninoculated pots (T_1).

Data analysis : Experiment was repeated twice and pooled data were analyzed using SPSS and subjected to ANOVA. Comparison among treatment means was done by Least Significance Difference using multiple mean-comparison test (Steel and Torrie, 1980).

Results and Discussion

Concentrated cultivation practices by and large depend on the extensive use of agrochemicals, which has resulted in serious environmental and health issues (Ongley *et al.,* 2010). Studies have shown that soil amendment with vermicompost enhances the performance of PGPR resulting in increased seed yield (Bajracharya and Rai, 2009; Singh *et al.,* 2012) and protection against pathogen in chickpea (Sahni *et al.,* 2008). However, most studies indicate individual effect of vermicompost, PGPR and salicylic acid on plant growth and development and induction of defense responses in chickpea (Sahni *et al.,* 2008; War *et al.,* 2011; Bhar *et al.,* 2018; Mazumdar *et al.,* 2019). In the present investigation, an attempt was made to determine the effect of vermicompost and PGPR isolate for improved plant growth. Further, the combined effect of salicylic acid, vermicompost and PGPR were analyzed for effective management of chickpea wilt disease.

Soil amended with varying amounts of vermicompost (10, 15, and 20%) progressively increased root and shoot length as well as fresh and dry weight of chickpea plants when compared to control plants (Fig. 1). However, the root length, shoot length, fresh weight, and dry weight of chickpea plants grown in soil amended with 20% vermicompost did not differ significantly from seedlings grown in soil amended with 15% vermicompost (Fig. 1). Interestingly, only seed bacterization with PGPR-WS resulted in a significant increase in root and shoot length, as well as fresh and dry weight, when compared to control plants and plants grown on 10 & 15% soil amended with vermicompost (Fig. 1). However, the collective treatments of PGPR-WS with vermicompost amendment significantly improved the above mentioned growth parameters of chickpea plants as compared to the control as well as the sum of their individual effects. It was observed that the combined use of 15% vermicompost along with PGPR-WS treatment showed ~ 43%, ~ 45%, ~ 50% and ~ 41% increase in root length, shoot length, fresh and dry weight, respectively, compared to control (Fig. 1). The enhancement of biomass production in vermicompost amended soil might be due to increased nutrient uptake and other beneficial effects like production of phytohormones (Frankenberger and Arshad, 1995) and phosphate solubilization activities (Atiyeh *et al.,* 2000; Sahni *et al.,* 2007; Kumar *et al.,* 2018). The reduction in root length, shoot length, fresh weight, and dry weight of chickpea plants at higher dose of vermicompost might be due to non-synchronization of nutrient release from the vermicompost and soil with plant nutrition (Sahni *et al.,* 2008).

The PGPR isolate (PGPR-WS) used in this study also showed high level of IAA production and phosphate solubilizing activity (Kumar *et al.,* 2018). The combined effect of vermicompost and PGPR on root and shoot length, and fresh and dry weight of chickpea plants were quite evident in the present study, which indicates the synergistic effect of both the treatments. Our findings clearly show that vermicompost aided the beneficial effects of PGPR (synergistic interactions) on root and shoot growth of chickpea plants. The previous report also supports our finding of synergistic effect of vermicompost and

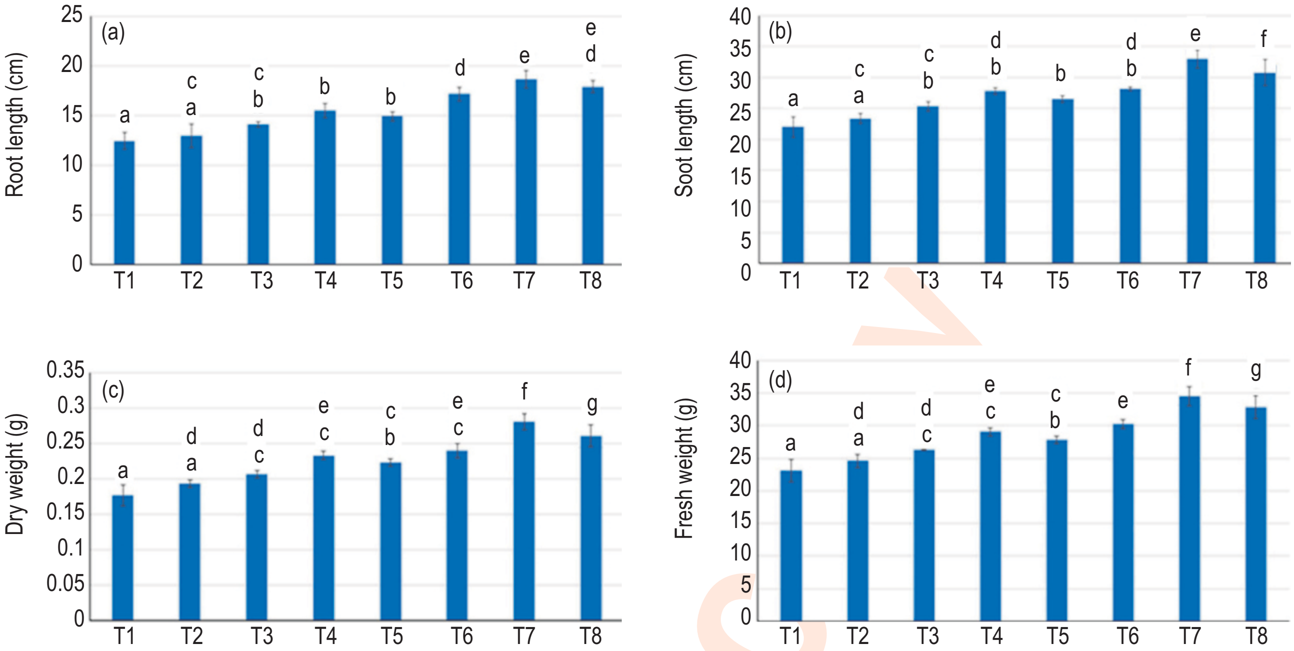

Fig. 1 : Effect of seed bacterization and different rates of vermicompost amendments alone and in combination on growth promotion of chickpea plants after 50 days. Treatments: T_1 = Control (soil), T_2 = vermicompost amendment @ 10% v/v, T_3 = vermicompost amendment @ 15% v/v, T_4 = vermicompost amendment @ 20% v/v, T_5 = seed bacterization with PGPR-WS, $T_6 = T_2 + T_5$, $T_7 = T_3 + T_5$ and $T_8 = T_4 + T_5$. Means, within the same column, superscript by the same letter are not significantly different ($P \leq 0.05$) by ANOVA-protected LSD test.

PGPR on growth and development of chickpea (Song *et al.,* 2015). Therefore, PGPR-WS can be used as additional inoculants in conjunction with vermicompost to create a synergistic treatment for improving root and shoot growth of chickpea.

Spraying $ZnSO_4$ and salicylic acid did not show any phototoxic effect on chickpea plants. However, all the treatments showed varied degrees of reduction in the wilt incidence compared to inoculated control which showed 46.67, 96.67 and 100 % wilt incidence after 10, 20 and 30 dpi, respectively. Among the single treatments of chemicals, the wilt incidence was highly reduced in chickpea plants sprayed with salicylic acid compared to $ZnSO_4$. The treatment of salicylic acid resulted in 53.33% wilt incidence while treatment of $ZnSO_4$ showed 76.67% wilt incidence at 30 dpi (Table 1). Since both chemicals were used at very low concentrations, the toxic effect of $ZnSO_4$ and salicylic acid on any known pathogens was not reported (Sahni *et al.,* 2008; Mandal and Sinha, 1992). According to earlier reports, the use of salicylic acid at lower concentrations was found to be safe for the chickpea plant and was used to induce antioxidant enzymes, resulting in an improved defence system (War *et al.,* 2011; Gayatridevi *et al.,* 2012; Boukraa *et al.,* 2017). It is well established that the application of salicylic acid causes the activation of systemic acquired resistance (SAR) in various crops (Gao *et al.*, 2015). Few crops have been reported to have salicylic acid-mediated resistance to *F. oxysporum* (Ojha *et al.*, 2012; Yousif, 2018). Foliar application of salicylic acid, which cross-talks and induces endogenous nitric oxide and reactive oxygen species, was recently shown to improve growth and defence responses against wilt pathogens in tomato plant (Chakraborty, 2021).

It has been reported that foliar application of chemicals such as H_3BO_3, $CuSO_4$, $MnCl_2$, $KMnO_4$ *etc.*, leads to activation of systemic acquired resistance, which inhibits the incidence of fungal diseases (Sarkar and Sinha, 1988; Reuveni and Reuveni, 1998). The efficacy of salicylic acid in controlling wilt disease over $ZnSO_4$ might be due to activation of systemic acquired resistance, an induced defence mechanism that provides long-term safeguard against a wide range of microorganisms, which leads to production of defense enzymes and pathogenesis related proteins. According to the European Union's harmonised classification and labelling (CLP00), $ZnSO_4$ is very toxic to aquatic life and causes serious eye damage (https://echa.europa.eu/brief-profile/-/briefprofile/100.028.904). Despite the fact that $ZnSO_4$ has several beneficial effects in terms of nutrient and disease management in crops (Pathak *et al.,* 2012; Deshlahare *et al.,* 2019; Sahni *et al.,* 2008), it should be used with caution in agriculture.

Vermicompost amended soil along with pre-inoculated foliar spray of chemicals, showed significant decline in chickpea wilt incidence gradually as compared to control as well as their

Table 1 : Effect of salicylic acid and $ZnSO_4$ alone and in combination with seed bacterization with PGPR-WS and vermicompost amendments on wilt incidence of chickpea plants caused by *F. oxysporum* f. sp. *ciceri.*

Treatments	Wilt incidence (%) ‡								
	Mock treatment			Zinc sulphate @ 50 ppm			Salicylic acid @ 40 ppm		
	10th day	20th day	30th day	10th day	20th day	30th day	10th day	20th day	30th day
T_1	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
T_2	46.67^{b}	96.67^{b}	100.00^{b}	36.67^{b}	70.00^{b}	76.67^{b}	30.00^{b}	50.00^{b}	53.33^{b}
T_3	30.00^{c}	66.67^{c}	73.33^{c}	26.67^{c}	53.33^{c}	56.67^{c}	23.33^{b}	43.33^{bc}	46.67^{bc}
	(16.67)	(30.00)	(26.67)	(10.00)	(16.67)	(20.00)	(6.67)	(6.67)	(6.66)
T_4	26.67^{cd}	60.00^{c}	66.67^{cd}	16.67^{d}	50.00^{c}	53.33^{c}	10.00^{c}	36.67^{cd}	40.00^{cd}
	(20.00)	(36.67)	(33.33)	(20.00)	(20.00)	(23.34)	(20.00)	(13.33)	(13.33)
T_5	20.00^{de}	56.67^{cd}	60.00^{de}	13.33^{d}	40.00^{cd}	43.33^{cd}	6.67^{acd}	33.33^{de}	36.67^{cde}
	(26.67)	(40.00)	(40.00)	(23.34)	(30.00)	(33.34)	(23.33)	(16.67)	(16.66)
T_6	13.33^{ef}	46.67^{de}	50.00^{ef}	10.00^{d}	33.33^{de}	36.67^{de}	3.33^{acde}	26.67^{e}	30.00^{def}
	(33.34)	(50.00)	(50.00)	(26.67)	(36.67)	(40.00)	(26.67)	(23.33)	(23.33)
T_7	6.67^{afg}	36.67^{ef}	43.33^{fg}	0.00^{a}	20.00^{ef}	26.67^{ef}	0.00^{adef}	13.33^{f}	20.00f
	(40.00)	(60.00)	(56.67)	(36.67)	(50.00)	(50.00)	(30.00)	(36.67)	(33.33)
T_8	3.33^{agh}	23.33^{g}	30.00^{h}	0.00^{a}	13.33^{afg}	13.33^{afg}	0.00^{adefg}	0.00^{ag}	0.00^{ag}
	(43.34)	(73.34)	(70.00)	(36.67)	(56.67)	(63.34)	(30.00)	(50.00)	(53.33)
T_9	3.33^{agh}	26.67^{fg}	33.33^{gh}	0.00^{a}	16.67^{fg}	20.00^{fg}	0.00^{adefg}	0.00^{ag}	3.33^{ag}
	(43.34)	(70.00)	(66.67)	(36.67)	(53.33)	(56.67)	(30.00)	(50.00)	(50.00)

Treatments: T_1= Control (uninoculated), T_2= Control (inoculated with *Fusarium oxysporum* f. sp. *ciceri*), T_3= Vermicompost amendment @ 10% v/v, T_4= Vermicompost amendment @ 15% v/v, T_5= Vermicompost amendment @ 20% v/v, T_6= Seed bacterization with PGPR-WS, T_7= T_3+ T_6, T_8= T_4+ T_6, and T_9= T_5+ T_6. ‡Means, within the same column, superscript by the same letter are not significantly different (Pd”0.05) by protected LSD test. The data in parentheses indicate percent reduction in wilt incidence over control (inoculated).

combined effects. The combined effect of vermicompost amendment @15% and pre-inoculation treatment of salicylic acid showed 0.00, 6.67 and 6.67% wilt incidence at 10, 20 and 30 dpi, respectively whereas treatments having $ZnSO_4$ as pre-inoculation foliar spray resulted in 0.00, 13.33 and 13.33% wilt incidence at 10, 20 and 30 dpi, respectively (Table 1). The results of this study clearly indicate the effectiveness of salicylic acid over $ZnSO_4$ in wilt disease inhibition (Table 1). Several reports advocate the use of soil amendment with vermicompost for easy nutrients uptake through plant's roots, enhancement of microbial activity in soil and also in fine tuning the defense enzymes produced in plants due to treatment of PGPR (Sahni *et al.,* 2007; Song *et al.,* 2015; Sahni and Prasad 2020).

Pseudomonas isolate (PGPR-WS) has previously been reported to exhibit multiple PGPR traits like ammonification, hydrogen cyanide production, indole acetic acid production, phosphate solubilisation and inhibition of growth of *F. oxysporum* f. sp. *ciceri* (75.00 %) which caused complete lysis of mycelia in advanced stages of antagonism (Kumar *et al.,* 2018). Treatment of PGPR-WS showed only 50 % wilt incidence at 30 dpi, but it was more effective when used in combination with foliar spray of salicylic acid or $ZnSO_4$, showing only 30.00 and 36.67% wilt incidence even at 30 dpi. Application of salicylic acid along with 20% vermicompost amendment or seed bacterization with PGPR-WS showed only 36.67 and 30.00% wilt incidence, respectively, at 30 dpi, which was not significantly different. Similarly, foliar spray of $ZnSO_4$ in combination with 20% vermicompost amendment and seed bacterization with PGPR-WS showed only 20% wilt incidence at 30 dpi whereas the same treatment along with foliar treatment of salicylic acid showed only 3.33% wilt incidence, which did not differ significantly from uninoculated healthy plant. The results clearly indicate the effectiveness of treatments when salicylic acid was used in combination with seed bacterization and vermicompost amendments. The combined treatment of 15% vermicompost along with seed bacterization and pre-inoculation foliar spray of salicylic acid showed complete protection against *F. oxysporum* f. sp. *ciceri* (Table 1). All treatments showed high wilt disease suppression when either salicylic acid or $ZnSO_4$ were applied to chickpea plants 24 hr before challenge inoculation with wilt pathogen. However, salicylic acid treatment showed maximum inhibition in all combination against *F. oxysporum* f. sp. *ciceri* compared to $ZnSO_4$. In our previous study, we have reported the management of collar rot of chickpea using chemicals like $ZnSO_4$ and oxalic acid, vermicompost and biological agent (*Pseudomonas syringae* strain PUR46) (Sahni *et al.,* 2008). Further, the expression of antioxident enzymes *viz.,* catalase, peroxidase and phenylalanine ammonia lyase were also found to be quite useful in management of collar rot of chickpea by integration of *Pseudomonas* sp. (PUR46) along with vermicompost (Sahni and Prasad, 2020). The combined application of vermicompost and seed bacterization with *Pseudomonas* sp. helped in maintaining the enhanced level of antioxidant enzymes, which indicates lesser requirement of defense enzymes for encountering the pathogen in chickpea (Sahni and Prasad, 2020).Taken together, introduction of biological agent which specifically reduced the growth of fungal pathogen along with salicylic acid and vermicompost were quite effective in the management of wilt disease in chickpea. This study demonstrated that salicylic acid, vermicompost, and PGPR have strong synergistic interactions for plant growth and dry matter accumulation, as well as developing eco-friendly management against chickpea wilt disease, indicating their utility in developing environmentally sound agriculture.

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Add-on Information

Authors' contribution: Sangita Sahni: Design the experiment, performed the research, analyzed the data and wrote the manuscript; **Sanjeev Kumar:** Performed the research and wrote methodology; **Bishun Deo Prasad**: analyzed the data and wrote the manuscript.

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