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Field efficacy of inorganic carrier based formulations of *Serratia entomophila* AB2 in *Sesamum indicum* var. Kanak

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Serratia entomophila **is a well-known bacterium of agricultural importance for its nutrient (P and Zn) solubilization, plant growth promoter (IAA) production, antifungal activity and larvaecidal activity against coleopteran and lepidopteron pest. In the present study, an attempt was taken to reduce the use of chemical pesticide and fertilizer by using two inorganic carriers (talcum powder and vermiculite) based formulation of *S. entomophila* in sesame. From the experimental results it was evident that the vermiculite based formulation of *S. entomophila* AB2 proved a better shelf life than that of talcum based formulation at 180th day. Mean-while, both of the inorganic carrier based formulations of *S. entomophila* AB2 showed better results than the unformulated *S. entomophila* AB2 product and 100% NPK (60:60:50). Experimental data ensured that vermiculite based formulation work more efficiently in controlling lepidopteron pest attack by 53% than that of talcum powder based formulation 31%. Productivity of sesame was also increased more with vermiculite based formulation (249%) in comparison to talcum powder based formulation (138%). It may be inferred that cumulative effect of high rate of seed germination, nutrient solubilization and reduced rate of pest attack resulted into 4.8 time enhanced yield in sesame in case of vermiculite formulated product. On the basis of the result of this experiment, it can be recommended that vermiculite (80 g/100 g of product) based formulation of *S. entomophila* AB2 applied at 3.6 qt hec^{-1} in sesame could be an effective measure of lepidopteron pest control as well as biofertilizer for qualitative and quantitative increase of sesame.**

Key words: *Serratia entomophila* AB2, formulation, talcum powder, vermiculite pest control, productivity, integrated crop management (ICM).

INTRODUCTION

Serratia entomophila is well known for its agricultural importance (Babalola, 2010). *S. entomophila* is mainly popular as natural biocontrolling agent of New Zealand grass grub (Coleoptera), *Costelytra zealandica* (Grimont et al., 1988). A Mexican strain of *S. entomophila* Mor.4.1 was reported to control white grub (Coleoptera), *Phyllophaga blanchardi* (Nunez-Valdez et al., 2008). *Serratia* sp. EML-SE1 was isolated in Korea from dead larva of diamondback moth (Lepidoptera), *Plutella xylostella* (Jeong et al., 2010).

Along with larvaecidal activity, *S. entomophila* was also explored for its multidimensional properties. *S. entomophila* was found to have significant contribution for increasing soil organic matter (SOM) and maintenance of soil ecology in pastures (Villalobos et al., 1997). Preconditioned cultures of *S. entomophila* were observed to survive better over untreated control in saline stress due to increased Glycine betaine and choline content (Sheen et al., 2013). *S. entomophila* M6 was demonstrated to neutralize heavy metals (Ji et al., 2012). Recently, genome sequencing projects have revealed great potential of *S. entomophila* as secondary metabolite producer (Bode, 2011).

The use of bacterial inoculants for agriculture is limited for a couple of reasons but the most notable among them is the poor efficacy of the product under field conditions (Prior, 1989). Acceptibility of the product largely depends on formulations of biopesticide or biofertilizer with increased selflife of microbial inoculant and efficient release for ensuring its subsequent availability to the target species. Scientists used different base compounds as suitable carrier to inoculate for formulation such as, talcum for fluorescent pseudomnads (Nandakumar et al., 2001); talcum and peat for *Pseudomonas chlororaphis* and *Bacillus subtilis* (Nakkeeran et al., 2004). An extended self life (8-10 months) with vermiculite based formulation was observed with *P. fluorescens* (Vidhyasekaran and Muthamilan, 1995) and *Azospirillum brasilense* (Saleh et al., 2001). The formulations of fluorescent *Pseudomonas* strain R62 and R81 were used to increase significantly plant growth and productivity in field condition (Sarma et al., 2009a). Broadcasting of talcum based formulation of *P. flurescens* strains (Pf1 and FP7) on paddy field significantly reduced sheath blight, and thereby, increasing yields (Nandakumar et al., 2001). Incorporation of commercial chitosan based formulation LS254 and LS255, comprising of *P. macerans* and *Bacillus subtilis* into soil at the ratio of 1:40 (Formulation:Soil) increased plant biomass and yield (Vasudevan et al., 2002).

The bacterial strain *S. entomophila* AB2, used in this study, was originally isolated from epizootic *Heliothis armigera* larvae (Chattopadhyay et al., 2011). The strain was characterized for nutrient (P and Zn) solubilization (Chattopadhyay et al., 2012a), plant growth promoter (IAA) production (Chattopadhyay and Sen, 2012b) along with antifungal (Chattopadhyay and Sen, 2013) and larvaecidal activity against lepidopteron pest. Studies on systemic infestation of this strain (Chattopadhyay and Sen, 2013) through plant parts encouraged its soil application. Therefore, the isolate *S. entomophila* AB2, as a single biological agent for integrated nutrient management (INM) and integrated pest disease management (IPDM) may have the potential to be a lucrative alternative to inorganic amendments (fertilizer, pesticides and fungicides) in integrated crop management (ICM) which need to be verified in field conditions. This communication makes an attempt to understand the feasibility of formulations involving a single indigenous strain, *S. entomophila* AB2 having multidimentional agricultural attributes for reducing the use of chemical pesticide and fertilizer in ICM practices. For this study, sesame was used as test crop. Two different inorganic carrier (talcum powder and vermiculite) based formulations were tested in field condition along with unformulated culture and NPK (60:60:50). Effectiveness of formulations was checked through a set of parameters: bacterial release in rhizosphere, self-life, pest control and productivity.

MATERIALS AND METHODS

Bacterial culture

The bacterial strain *S. entomophila* AB2, used in this study was isolated from epizootic *H. armigera* larvae (Chattopadhyay et al., 2011). The 16S rRNA gene sequence was registered to Gene Bank (Accession no. GU370899). The strain was characterized for nutrient (P and Zn) solubilization (Chattopadhyay et al., 2012a), plant growth promoter (IAA) production (Chattopadhyay and Sen, 2012b) along with antifungal (Chattopadhyay and Sen, 2013) and larvaecidal activity against lepidopteron pest.

Fermentation condition

Bacterial culture was maintained at -20°C as glycerol stock (50%). The working strain was grown in 100 ml flask containing broth medium (4 g sucrose, 1 g yeast extract, 0.2 g urea and 0.2 g NPK; pH 7.1) as seed culture. Fermentation was carried at 28°C for 72 h in a glass fermenter (MCU-200, B. Braun Biotech International, India) at 240 rpm using the same medium. Cells were harvested after entering into stationary growth phase (Visnovsky et al., 2008).

Product formulation

For product formulation two different inorganic carrier were used: talcum powder (TP; magnesium silicate, $Mg_3Si_4O_{10}(OH)_2$) and vermiculite (Ver; Phyllosilicate, $(MgFe,Al)_3(Al,Si)_4O_{10}(OH)_2 \cdot 4H_2O$). Sodium salt of carboxymethyl cellulose (CMC) was added in the formulations as an adhesive agent. After 3rd repeat sterilization, 80 g of carrier material was mixed with 18 ml of fermented broth (1.5×10^{10} cfu ml^{-1}), glycerol (1 ml 50% v/v) and CMC solution (1 ml 0.1 mg ml^{-1}) aseptically to generate 100 g of product (Vidhyasekaran and Muthamilan, 1995). The formulation was dried aseptically under the shade to reduce the moisture content to approximately

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Table 1. Product formulation and field trial experiments.

Test sample	Formulation	Field treatment
TS1	Control	Untreated
TS2	100% NPK (60:60:50)	100% NPK provided
TS3	90 ml fermented broth (*S. entomophila* AB2, 1.5×10^{10} cfu ml^{-1}) + 5 kg sterilized powdered soil	5 kg broadcasted in one plot area (4.0 m × 3.5 m)
TS4	18 ml fermented broth (*S. entomophila* AB2, 1.5×10^{10} cfu ml^{-1}) + 1 ml glycerol (50%, v/v) + 1 ml CMC (0.1 mg ml^{-1}) + 80 g talcum powder	500 g broadcasted in one plot area (4.0 m × 3.5 m)
TS5	18 ml fermented broth (*S. entomophila* AB2, 1.5×10^{10} cfu ml^{-1}) + 1 ml glycerol (50%, v/v) + 1 ml CMC (0.1 mg ml^{-1}) + 80 g vermiculite	500 g broadcasted in one plot area (4.0 m × 3.5 m)

18% and packed in sterilized polythene bags. The formulation contained 3.5×10^8 cfu g^{-1} of experimental bacterial load when packed. Formulation details are given in Table 1.

Field trials

Field trial experiments were conducted in three consecutive Ravi seasons. Experimental plots were kept idle for 6 months prior to seed sowing, to avoid the effects of any pesticide, chemical or biological, or any other application for soil treatment. The field soil was brought to a fine tilt by ploughing and 3.5 × 4.0 m plots were laid out. Randomized complete block design (RCBD) model was followed for the experiments.

Untreated (TS1) experimental plots were maintained as control whereas; other plots treated with 60:60:50 NPK (TS2). Unformulated experimental strain (TS3, 90 ml 1.5×10^{10} cfu ml^{-1}) cultures mixed with 5 Kg of powdered soil to broadcast over one plot area (4.0 × 3.5 m). For talcum powder based (TS4) and vermiculite based (TS5) formulations, 500 g of formulated product mixed with 4.5 kg of powdered soil to broadcast over one plot area. Treatment details were listed in Table 1. All experimental plots were irrigated, as per requirement to maintain the moisture level at 15%. In each case, broadcasting was done 1 h before sunset (Ghidiu and Zehender, 1993). After preparation of the field, surface sterilized seeds of sesame (*Sesamum indicum* var. Kanak) were sowed. Row to row distance was maintained at 30 cm whereas plant to plant distance was maintained 20 cm.

Inoculant availability assessment

After treatment, 1 g of soil of each treatment from day 10 and of intervals were suspended in 9.9 ml extraction buffer in tubes, containing 0.1% (w/v) tetra-sodium pyrophosphate and Tween 80 as an aid for proper cell dispersal. The tube containing sample was vortexed for 30 sec and placed inclined in an orbital shaker for 1 h at 10 rpm. The serially diluted sample was plated onto caprylate thallous agar (CTA) medium (O'Callaghan et al., 2002) supplemented with antibiotic Ampicillin (A) and Gentamicin (G) to measure the viable *S. entomophila* AB2 population.

Product self life assessment

For enumeration of viable inoculants from packed formulations same procedure was followed, at intervals from day 10. The serially diluted sample was plated onto caprylate thallous agar (CTA) medium (O'Callaghan et al., 2002) supplemented with antibiotic Ampicillin (A) and Gentamicin (G) to measure the viable AB2 population.

Pest control assessment

Experiments were carried out in open fields and therefore infested by different pest naturally. Only larvae of lepidopteron pests, particularly *H. armigera*, *Spodoptera litura* and *P. xylostella* were enumerated. Pest scouting was done in every alternative day after starting of fruit set and was continued up to harvesting. Total number of larvae was considered. Pest scouting was done in three consecutive Ravi season along with the field trial experiments.

Productivity assessment

For productivity assessment rate of seed germination (SG), growth parameters (average measurement of branch number (BN); shoot length (SL); shoot weight (SW) per plant) and yield parameters (average pod number/plant (PN); seed number/pod (SN); seed yield/plot (SY) were measured. The plants were air dried for a period of 7 days for measuring dry weight.

Statistical analysis

Standard deviation for each treatment was determined. The experimental data were statistically analyzed using ANOVA. Duncan's multiple range test (DMRT) was used to determine group mean value when ANOVA found significance at $P < 0.05$. Pesticidal activity was evaluated, through pest scouting and mortality rate evaluation, on the basis of severity of infestations (Amer et al., 1999).

RESLTS AND DISCUSSION

Effect of formulations on inoculant availability at rhizosphere

There was a significant difference in the viable count of inoculant from soil samples of TS3 with TS4 and TS5 (Figure 1). As found at day 10, soil treated with TS3 showed maximum count of viable inoculant (2.5×10^6 cfu

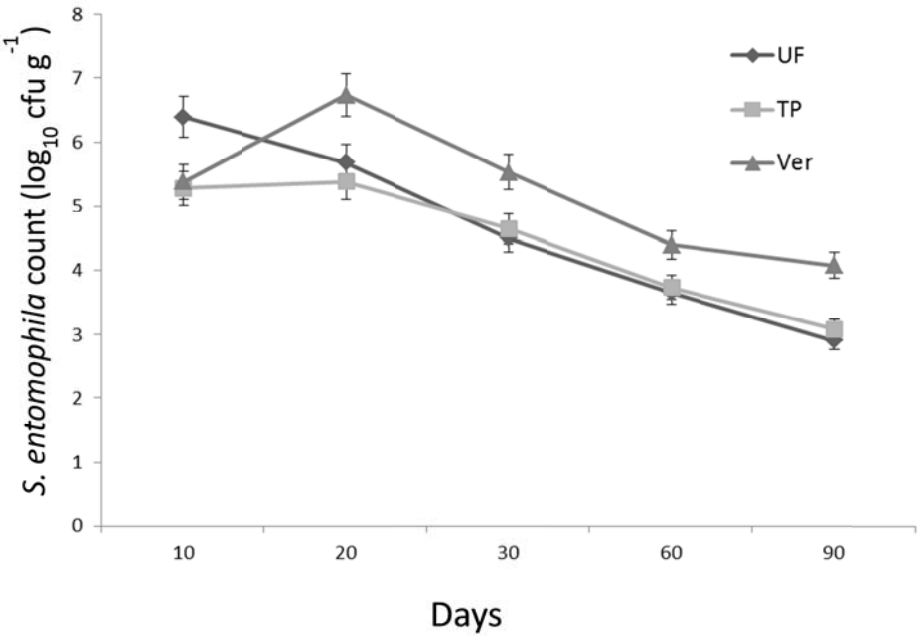

Figure 1. Efficacy of the different formulations of working isolate (UF, unformulated; TP, talcum powder based formulation; Ver, vermiculite based formulation) to release the bacterial isolates into the rhizosphere.

g^{-1}), in comparison to formulated samples TS4 (1.9×10^5 cfu g^{-1}) or TS5 (2.4×10^5 cfu g^{-1}) whereas, at day 20 the inoculant count from soil of TS3 was low (4.8×10^5 cfu g^{-1}), but higher while treated with TS4 (2.4×10^5 cfu g^{-1}) or TS5 (5.5×10^6 cfu g^{-1}). Thus, the results indicated slow release of inoculant from formulations and the vermiculite based formulation (TS5) was found to release microbial inoculant more efficiently. However, a gradual decrease of inoculant count from soil was observed thereafter.

Standardization of formulation is a challenging part and often a success limiting step in development of biocontrol products (Paau, 1998). It becomes further critical, if the active microorganism is non-spore former (O'Callaghan and Gererd, 2005). Successful release of *S. entomophila* was recorded at various soil moisture level while worked with clay based prill (O'Callaghan et al., 2002) or granule (O'Callaghan and Gererd, 2005). In the present study, *S. entomophila* AB2 population in rizosphere declined slowly with formulated samples in comparison to unformulated one. Vermiculite based formulation indicated more sustenance of inoculants.

Effect of formulations on inoculant self life

To determine the shelf life of the formulations, viable inoculant count of stored TS4 and TS5 products were estimated at monthly interval up to six months (Figure 2). Both the formulations had an initial bacterial loading of 3.5×10^8 cfu g^{-1}. On 30^{th} day, it decreased to 3.1×10^8 cfu g^{-1}. But, at 90 days of storage, inoculant load dropped by 5-fold and 10-fold in vermiculite and talcum based formulations respectively. The declining trend was observed thereafter up to the study period (6 month). It was evident that vermiculite based formulation of *S. entomophila* AB2 showed a better self life (3.6×10^6 cfu g^{-1}) than that of talcum based formulation (2.4×10^4 cfu g^{-1}) at 180^{th} day.

While soil was inoculated with unformulated *S. entomophila* 626, it was found that the rate of population decline increased with soil temperature though populations remained above the minimum level of detection after three months and soil moisture had little effect on survival (O'Callaghan et al., 2002). A biopesticide, containing a high density culture of the *S. entomophila* (Invade™), has been developed for control of grass grub in New Zealand (Jackson et al., 1992). But the liquid Invade™ product required to be maintained under refrigeration to avoid cell death during storage (Jackson et al., 1992). To overcome this problem Johnson et al. (2001) developed a system for stabilizing the bacterium in a biopolymer matrix, which can then be incorporated into clay-based granules. Later on, *S. entomophila* has been incurporated into prill formulations to improve distribution and application to pasture (O'Callaghan et al., 2002). Measurement of release of *S. entomophila* from prills in soils subjected to various watering regimes demonstrated that free soil water is important for distributing bacterial

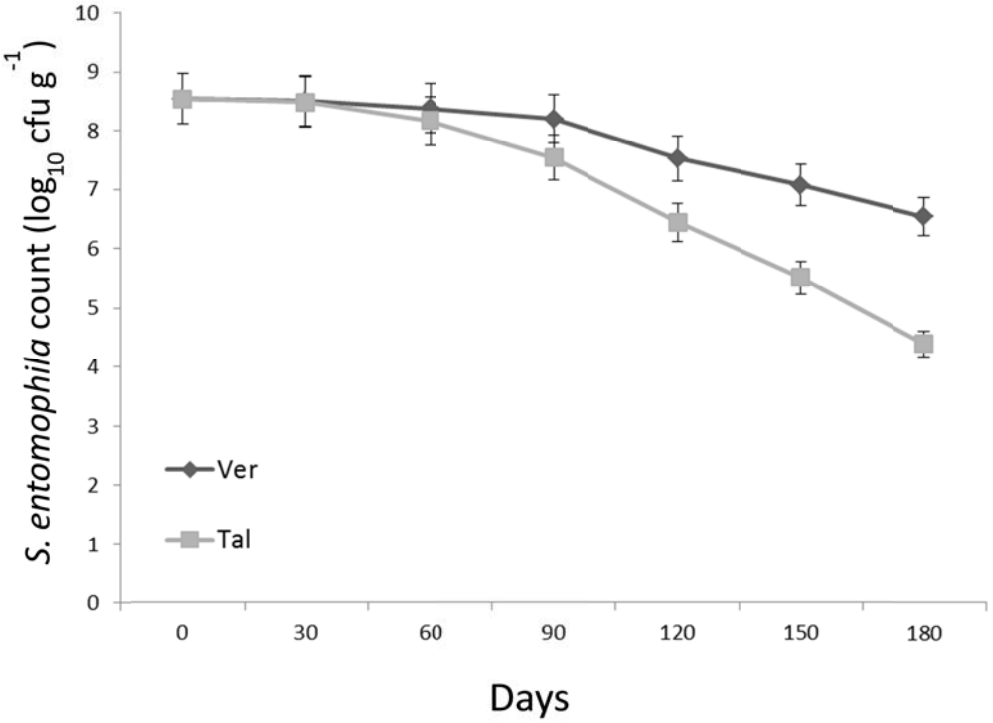

Figure 2. Self life of the two different formulations of working isolate (TP, talcum powder based formulation; Ver, vermiculite based formulation).

inoculum throughout soil profile. Another granular formulation of *S. entomophila* (Bioshield™) was developed by Townsend et al. (2004). In the present study vermiculite based formulation of the working strain *S. entomophila* AB2 ensured its significant viability up to experimental period of 6 months.

Effect on pest control

Highest pest attack was evident in 100% NPK (60:60:50) (TS2) which was found 115% more in comparison to control (TS1). A significant decrease in pest scouting (119%) was observed in plots treated with unformulated strain (TS3) in comparison to control (TS1). Experimental data ensured that vermiculite based formulation work more efficiently in minimizing lepidopteron pest (*H. armigera* 45%, *S. litura* 50%, *P. xylostella* 53%) than that of talcum powder based formulation (*H. armigera* 30%, *S. litura* 44%, *P. xylostella* 31%) (Figure 3).

It was reported that broadcasting of talcum based formulation of *P. flurescens* strains (Pf1 and FP7) on paddy field significantly reduced sheath blight, thereby, increasing yield (Nandakumar et al., 2001). Similarly, the present study clearly demonstrates, that even the plot treated with *S. entomophila* AB2 alone (TS3) can provide an effective measure for controlling lepidopteron pest infection.

Effect on productivity

The rate of seed germination in different soil treatments was observed (Figure 4). It was found that the rate was much low in TS1 (73.8%), TS2 (81.8%) and TS3 (83.8%) than formulations TS4 and TS5 showing almost 100% germination (97.4%). The profound effect of plant growth was recorded in terms of BN, SL and SW upon treatment with *S. entomophila* AB2 formulation (Figure 4). Particularly, the vermiculite based formulation (TS5) showed maximum effect and the increment was recorded in SL (155.54%), SW (218.87%) over the control (TS1). From the experimental data, it was also evident that productivity was more with vermiculite based formulation (SW 78%, SY 138%) than that of talcum powder based formulation (SW 119%, SY 249%) except SG in comparison with control. Cumulative effect of high rate of seed germination, reduced rate of pest attack resulted to 4.8 time enhancement of yield in sesame (Figure 4).

Effect of microbial consortium for seed germination is well studied (Pandey and Maheshwari, 2007; Babalola et al., 2007; Chen and Nelson, 2008; Naik and Sreenivasa, 2009). Formulations of *Pseudomonas* through application of the working isolate *S. entomophila* showed significant increase of seed germination in *Vigna mungo* (Sarma et al., 2009a). Similar trend was achieved AB2 in seed germination experiment. From earlier reports formulations of fluorescent *Pseudomonas* strain R62 and R81 were

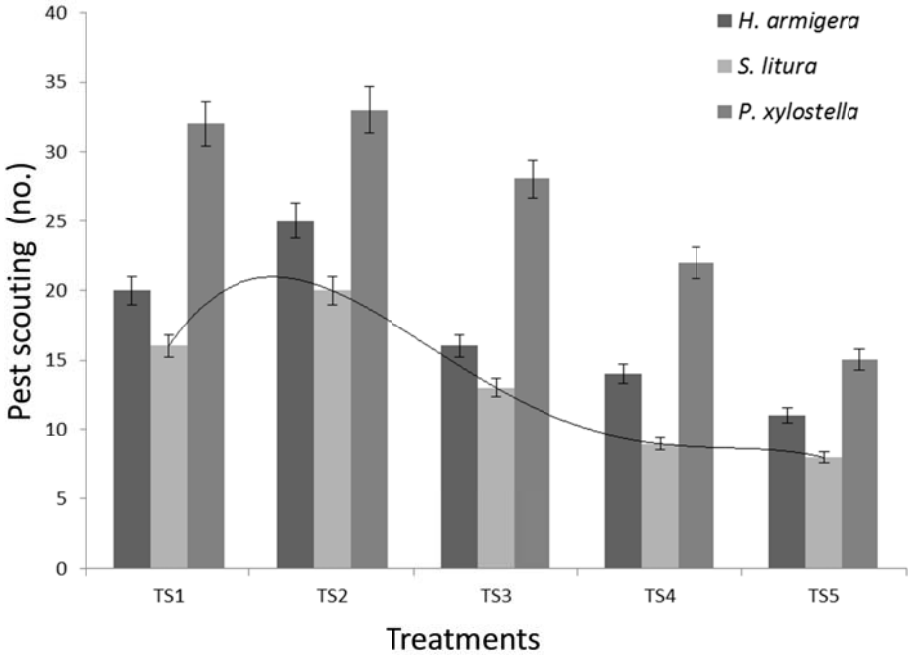

Figure 3. Effect of field treatment with control (TS1), 60:60:50 NPK (TS2), unformulated strain (TS3), talcum powder based formulation (TS4**)** and vermiculite based formulation (TS5) on providing protection against lepidopteron pest**s**.

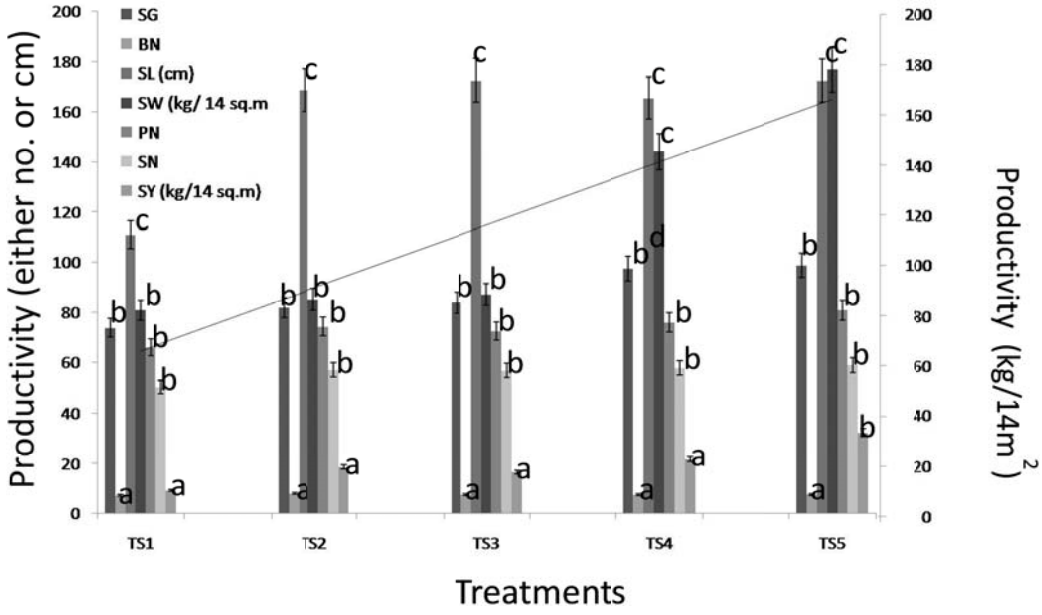

Figure 4. Effect of field treatment with control (TS1), 60:60:50 NPK (TS2), unformulated strain (TS3), talcum powder based formulation (TS4) and vermiculite based formulation (TS5) on productivity in terms of seed germination (SG), branch number (BN), shoot length (SL), shoot weight (SW), pod number (PN), seed number (SN) and seed yield (SY).

known to increase plant growth and producti-vity signi-ficantly in field condition (Sarma et al., 2009b). Since, the isolate *S. entomophila* AB2 was found to solu-bilize macro- and micro-nutrients (P and Zn)(Chattopadhyay et

al., 2011) it could be assumed that the nutrient availability was reflected in productivity.

Conclusion

The strain *S. entomophila* AB2, as a single biological agent for INM and IPDM seems to be a lucrative alternative to chemical fertilizer, pesticides and fungicides in ICM. The present study describes field trial of *S. entomophila* AB2 through inorganic carrier formulations, as soil inoculant. In addition to maintain its self life, the vermiculite based formulation can enhance field efficacy by improving establishment of microbial inoculant in soil microenvironment. Cumulative effect of high rate of seed germination, reduced rate of pest attack resulted into 4.8 time enhancement of yield in sesame. On the basis of the result of this study it can be recommended that vermiculite (80 g/100 g of product) based formulation of *S. entomophila* AB2 could be used at the rate of 3.6 qt hec^{-1} for quality and yield improvement of sesame. The information presented here may otherwise be useful for rice, pulse and cotton crops, where lepidopteron pest like *S. litura* (cutworm), *H. armigera* (bollworm) and *P. xylostella* (diamond back moth) outbreaks are common.

Conflict of Interests

The author(s) have declared no conflict of interests.

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