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# Exploration of *Serratia entomophila* AB2 for lepidopteran pest control and productivity of groundnut

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**The bacterial isolate *Serratia entomophila* AB2 having multidimensional attributes like larvacidal activity and nutrient-solubilizing capability was used to test its effect on productivity of groundnut. Two different inorganic carriers (talcum powder and vermiculite) based formulations of the bacterial isolate were also tested along with the unformulated product and 100% recommended dose of NPK (60:60:50) for comparative study. *S. entomophila* AB2 (unformulated) efficiently minimized lepidopteran pest infestations when compared with control (larval stage of *Heliothis armigera,* 27%; *Spodoptera litura*, 30%; *Plutella xylostella,* 23%). The strain also increased seed germination by 10%, increment in shoot weight was by 100% and enhancement of seed weight was by 120% in comparison to negative control in a field trial. But, both of the inorganic carrier based formulations showed better results in respect to pest control and productivity compared to the unformulated bacterium. Among the two formulations used in this study, vermiculite based formulation was found effective in terms of pest control and productivity. Consequently, a vermiculite based formulation of *S. entomophila* AB2 could be effectively used at the rate of 3.6 qthec$^{-1}$ for quality and yield enhancement of groundnut.**

**Key words:** *Serratia entomophila* AB2, fermentation, pesticide, nutrient solubilizer, integrated crop management (ICM) productivity.

## INTRODUCTION

Several, bacterial entomopathogens have been developed for their use as commercial pest controlling agent and are *Bacillus thuringiensis*, *B. cereus*, *Burkholderia cepacia*, *Serratia entomophila*, *Pseudomonas fluorescens* (Johnson et al., 2001; Roh et al., 2009; Jeong et al. 2010; Sheen et al., 2013). But application of bacterial entomopathogens as soil inoculants is of rare occurrence. Among bacterial biopesticides *S. entomophila* was commercialized as soil inoculants to control pasture pest *Costelytra zealandica* (Coleoptera) in New Zealand (Johnson et al., 2001).

The strain *S. entomophila* AB2, used in this study, was reported with two unique features. Instead of controlling coleopteron pasture pests, *S. entomophila* AB2 was reported to control lepidopteron pests (*Heliothisarmigera*, *Spodopteralitura*, *Plutellaxylostella*) of phyllosphere region (Chattopadhyay et al., 2011, Chattopadhyay and Sen, 2012)

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| | | | | | |
|---|---|---|---|---|---|
| R1 | TS1 | TS3 | TS4 | TS2 | TS5 |
| R2 | TS2 | TS3 | TS1 | TS5 | TS4 |
| R3 | TS1 | TS5 | TS2 | TS4 | TS3 |
| R4 | TS5 | TS4 | TS3 | TS2 | TS1 |

**Figure 1.** Randomized complete block design (RCBD): Experimental field showing different treatments (TS1, TS2, TS3, TS4 and TS5) with four replications (R1, R2, R3 and R4).

The strain was also characterized for P and Zn solubilization property (Chattopadhyay and Sen, 2012). Microbial organisms that solubilize nutrients is an important option for integrated crop management (ICM) that help to nourish the crop plants by increasing availability of soil nutrients (Gyaneshwar et al., 2002). Studies also proved systemic infestation of *S. entomophila* AB2 through plant parts to provide protection against pest and pathogen (Chattopadhyay and Sen, 2013). Therefore, the strain *S. entomophila* AB2 could be used in agriculture as potential pesticide (because of larvaecidal actiivity) and fertilizer (because of nutrient solubilization).

Limiting factors of using bacterial inoculants in field are related to storage, distribution of the product and requirement of large volume of water for application as suspension (Sabbour et al., 2012). Further, the use of biopesticide is limited for a couple of reasons but the most notable among them is the poor efficacy of the product under treatment conditions (Prior, 1989; Sabbour et al., 2012). To overcome these limitations, multitasking isolates with new formulations having extended shelf life and easy application are required to be developed.

In the present study, agricultural potentiality of *S. entomophila* AB2 was tested against groundnut, an important oil yielding cash crop highly susceptible to pest and pathogens (Adjou et al., 2012). Two different inorganic carriers (talcum powder and vermiculite) were tested for formulation. This communication makes the first attempt to understand the feasibility of a single indigenous strain, *S. entomophila* AB2, for pest control and productivity assessment in field condition for reducing the use of chemical pesticide and fertilizer.

## MATERIALS AND METHODS

### Experimental strain

The bacterial strain *S. entomophila* AB2 reported for its nutrient solubilizing, antifungal and larvicidal activity (Chattopadhyay et al., 2011; Chattopadhyay and Sen, 2012, 2013) was used. The 16S rRNA gene sequence was registered to Gene Bank (Accession no. GU370899). The isolate was maintained as 50% glycerol stock at -20°C in brain heart infusion agar (BHI-agar, Hi-media, India).

### Fermentation

The working strain was grown in shake flask using 100 ml of broth (4 g sugar, 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 in same medium considering other parameters as per Visnovsky et al. (2008). Cells were harvested after they had entered the stationary growth phase.

### Inorganic carrier used

For product formulation, talcum powder (magnesium silicate, $Mg_3Si_4O_{10}(OH)_2$) and vermiculite (Phyllosilicate, $(MgFe,Al)_3(Al,Si)_4O_{10}(OH)_2 \cdot 4H_2O$) were used as inorganic carriers. Sodium salt of carboxymethyl cellulose (CMC), generally referred as cellulose gum, was added as an adhesive agent.

### Product formulation

After repeated sterilization, 80 g of carrier material was mixed with 18 ml of fermented broth (1.5 x $10^{10}$ cfu $ml^{-1}$), glycerol (1 ml, 50% v/v) and CMC solution (1 ml, 0.1 mg $ml^{-1}$) aseptically and uniformly to generate 100 g of product (Vidhyasekaran and Muthamilan, 1995). The formulation was shade dried aseptically to reduce the moisture content to ~18% and packed with in sterilized polythene bags under UV radiation and sealed. The formulation contained 3.5 x $10^8$ $cfug^{-1}$ of experimental bacterial load when packed. 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 from the packed formulation at intervals of 10 days.

### Field trials

Soil conditioning experiments were conducted in Ravi season in experimental field (red laterite soil), keeping the field idle for 6 months prior to seed sowing for avoiding effects of any pesticide. Plots of 3.5 m x 4.0 m were laid out and brought to a fine tilt by ploughing. Soils of the plots were mixed well ensuring leveling and rows were made in 30 cm apart. Randomized complete block design (RCBD) model was followed for the experiments (Figure 1). Untreated (TS1) experimental plots were taken as control, whereas other plots supplied with 100% recommended dose of NPK (60:60:50) (TS2) were also maintained. Plots with soil conditioning was done with unformulated experimental strain (TS3), 90 ml 1.5 x $10^{10}$cfu $ml^{-1}$ cultures mixed with 5 Kg of powdered soil to broadcast over one plot area (4.0 m x 3.5 m). For talcum powder based (TS4) and vermiculite based (TS5) formulations, 500 g of formulated product (having 3.5 x $10^8$ $cfug^{-1}$) was mixed with 4.5 kg of powdered soil to broadcast over one plot area (4.0 m x 3.5 m). All experimental plots were irrigated, as required to maintain the moisture level at 15%. In each case, treatment was carried out one hour before sunset (Ghidiu and Zehender, 1993).

### Seed germination assessment

Seeds of ground nut (*Arachis hypogaea* var. Koushal, G201) were

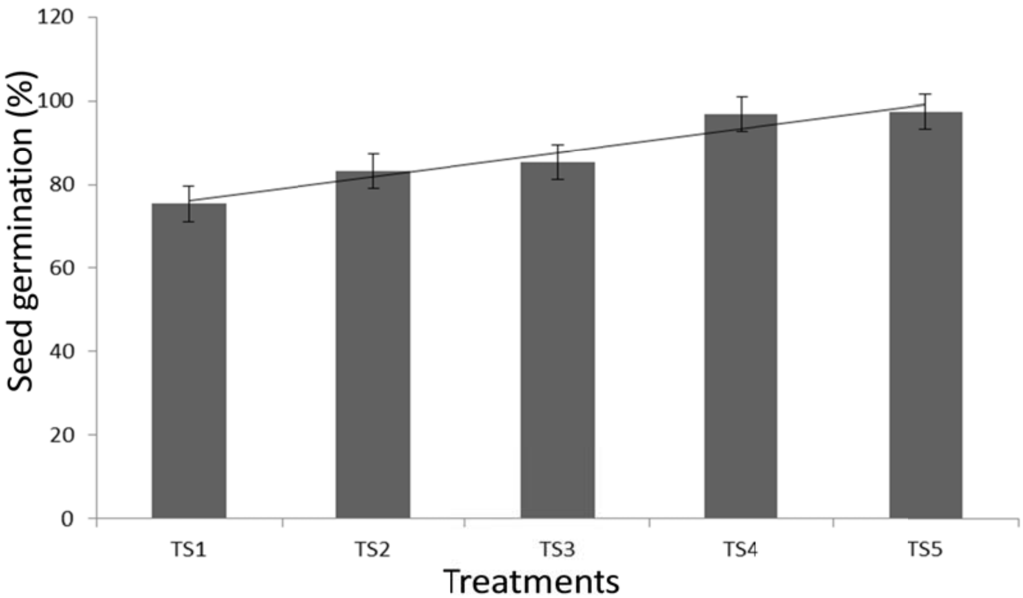


**Figure 2.** Effect of field treatment with, control (TS1), 100% NPK (TS2), unformulated strain (TS3), talcum powder based formulation (TS4) and vermiculite based formulation (TS5) on seed germination of ground nut.

surface sterilized and soaked in distilled water for 10 min and sowed in rows at a distance of 20 cm between the two as shown in Figure 1. After 10 days of sowing of seeds, seed germination percentage was recorded.

### Pest control assessment

Experiments were carried out in open fields; therefore, the experimental field could have been infested by different pest naturally. Only larvae of lepidopteran pests, particularly *Heliothis armigera*, *Spodoptera litura* and *Plutella xylostella* were enumerated because the experimental strain is a known pathogen to these pest species (Chattopadhyay and Sen, 2012). Data was recorded since the 30$^{th}$ day of seed sowing till the period of experiment.

### Productivity assessment

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

### Statistical analysis

Standard deviation for each treatment was determined from four replications. The experimental data were statistically analyzed using ANOVA. Duncan's multiple range test (DMRT) was used to determine group mean value when ANOVA was found significant 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).

## RESULTS AND DISCUSSION

### Effect on seed germination

The rate of seed germination in different soil treatments was observed (Figure 2). It was found that the rate was much low in TS1 (75.4%) and in TS 2 (83.2%) While seeds of TS3 (85.4%) showed lower rate of germination than formulations (TS4 and TS5), causing almost 100% germination (96.8 and 97.4% respectively).

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* was used and showed significant increase of seed germination in *Vigna mungo* (Sarma et al., 2009a). Similar trend was achieved through application of the working isolate *S. entomophila* AB2 in seed germination of ground nut.

### Effect on pest control

Highest pest attack was evident in plots treated with NPK (60:60:50) (TS2) which was found 119.56% more in comparison to control (TS1) (Figure 3). The results showed that *S. entomophila* AB2 (unformulated) efficiently minimized lepidopteron pest infestations when compared with control (*Heliothisarmigera,* 27%; *Spodopteralitura,* 30%; *Plutellaxylostella,* 23%). Among the experimental plots least pest attack was found in vermiculite based formulation of *S. entomophila* AB2 (TS5) (176.92% less pest attack thanTS2).

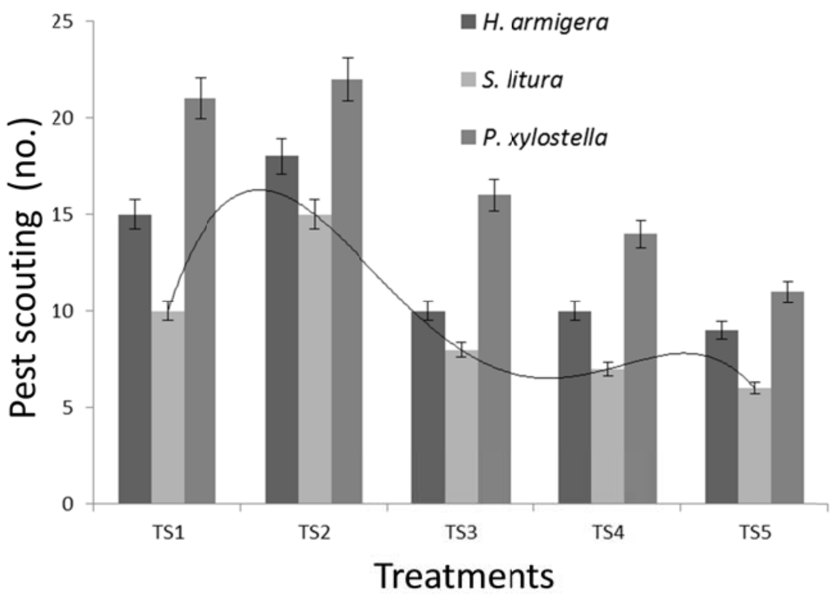


**Figure 3.** Effect of field treatments with control (TS1), 100% NPK (TS2), unformulated strain (TS3), talcum powder based formulation (TS4) and vermiculite based formulation (TS5) on providing protection against lepidopteran pests.

**Table 1**. Effect of *S. entomophila* AB2 and its formulations on productivity of ground nut.

| Treatment | Branch number | Shoot length (cm) | Dry shoot weight (kg per experimental plot) | Pod number per plant | Seed number per pod | Seed yield (kg per experimental plot) |
|---|---|---|---|---|---|---|
| TS1 (control) | 3.8±0.2 | 55.33±0.4 | 56.45±0.4 | 12.8±0.3 | 1.27±0.05 | 16.44±0.3 |
| TS2(100% NPK) | 6±0.3 | 61.33±0.5 | 70.2±0.7 | 20.5±0.3 | 1.9±0.05 | 40.08±0.4 |
| TS3(unformulated strain) | 4.5±0.25 | 63±0.5 | 70.23±0.7 | 18.9±0.3 | 1.5±0.05 | 35.66±0.4 |
| TS4(talcum powder based formulation) | 5.7±0.3 | 65±0.5 | 104.46±0.7 | 21.93±0.3 | 2.1±0.05 | 42.83±0.4 |
| TS5 (vermiculite based formulation) | 4.1±0.2 | 71.6±0.7 | 138.12±0.8 | 25.1±0.3 | 2.25±0.06 | 62.89±0.5 |

The value were presented as mean value of four replication ± standard error.

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 demonstrated that, even the soil treated with *S. entomophila* AB2 alone (TS3) can provide an effective measure for controlling lepidopteron pest infection.

## Effect on productivity

The observations recorded on plant growth in terms of BN, SL and SW, clearly indicated positive effect of the *S. entomophila* AB2 (Table 1). The strain (TS3) alone worked efficiently by 100% increment in shoot weight and 120% enhancement of seed weight in comparison to control in field trial. In growth experiments, both the formulations (TS4 and TS5) showed profound effect. Particularly, the vermiculite based formulation (TS5) showed maximum effect (129.47% increment in shoot length and 244.67% increment in shoot weight, in comparison to the control). Further, the SY was found maximum with vermiculite based formulation (TS5) and 156.91% increment in ground nut production was achieved in comparison to TS2 where soil was treated with NPK (60:60:50) (TS2).

The results show that the yield criteria viz., PN, SN and SY were influenced by different treatments TS5>TS4>TS2>TS3>TS1. It indicates that formulations may be required for better availability of the strain in the rhizosphere for longer time duration. Thus, the effective role of *S. entomophila* AB2 on growth and yield parameters was evident. From earlier reports formulations of fluorescent *Pseudomonas* strain R62 and R81 were known to increase plant growth and productivity significantly in field condition (Sarma et al., 2009b).

## Conclusion

The strain *S. entomophila* AB2, as a single biological agent for integrated nutrient management (INM) and integrated pest and disease management (IPDM) may find its application as a lucrative alternative to chemical fertilizer, pesticides and fungicides in ICM. But before that, its field efficacy should be checked. In the present study results of the field application indicate that the strain could be used as a soil inoculant. However formulations may be required for better availability of the strain in the rhizosphere for longer time duration which requires further investigation. On the basis of the result of this study, it can be recommended that vermiculite based formulation of *S. entomophila* AB2 could be used at the rate of 36 kg per 1000 sqm (3.6 qt $hec^{-1}$) for better quality and yield in groundnut. This could be a remunerative recommendation as it could effectively reduce the cost of chemical fertilizers and pesticides.

## Conflict of Interests

The authors have not declared any conflict of interests.

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