Field evaluation of native white grub bio-agent, *Bacillus cereus* strain WGPSB-2 in Uttarakhand Himalayas and its impact on soil microbiota

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Abstract

A microbial pathogen, *Bacillus cereus* strain WGPSB-2 isolated from mid hills of Uttarakhand Himalayas (1000-1500 amsl) had potential as a biocontrol agent against white grubs of the region. Thus, the pathogen’s ability to suppress grubs was investigated under field conditions over a period of five years in five villages of the region. A single dose annual application of WGPSB-2 as tål based formulation (1x10^8 spores/g) at the rate of 5kg/ha showed a sharp decline in grub population over the years in all the targeted villages. Pit sampling data showed maximum grub reduction during initial years of treatment in all villages and found significant for time, villages and time X village interactions in repeated measures ANOVA. Twenty four months post-inoculation resilience took place in micropits tested for bioagent impact on micro-floral densities, resulting in similar/ positive structures of tested microbial populations. The substantial numerical reduction of grub density coupled with no negative impacts on soil inhabitant microbiota, signifies WGPSB-2 as a vibrant technological option in this ecologically sensitive region.

Keywords: *Bacillus cereus*, management, microbiota, Uttarakhand Himalayas, white grubs

Introduction

“White grub” is a collective term used for root feeding scarab larvae of the families Scarabaeidae, Dynastidae, Rutelidae and Cerambycidae in order Coleoptera. They cause significant damage to many agricultural and horticultural crops, ornamentals, plantation crops, lawn, turf, pasture and forest trees around the world and are major limiting factor of agricultural production in India [17]. Though white grub incidence has been documented in different agro-ecological regions of India, their prevalence is highly pronounced in the North-Western Indian Himalayan region comprising the states and union territories of Uttarakhand, Himachal Pradesh, Jammu and Kashmir and Ladakh. As per available records, this region harbours nearly 78 species of phytophagous white grub species [19]. However, the mid hills (1000-1500 amsl) of Uttarakhand, where the study was undertaken harbour Anomala dimidia (Hope), *H. seticollis* Moser and *H. longipennis* as predominant species [16, 18].

Keeping in view, the environmental contamination by chemical pesticides especially in the dynamic hilly ecosystem, biocontrol can be considered as ecofriendly alternative [4, 12]. The association of variety of native microbial entomopathogens with white grubs, innate hardiness to prevailing climate [7], their governance in natural regulation of pest [21] etc offers promises in successful pest management. Moreover, in order to formulate new biological control programme, evaluation of the bio-agent interacting dynamics among the entomopathogenic organism, host and environment is necessary [2].

Our exploration studies yielded an entomopathogenic bacterium, *Bacillus cereus*, strain WGPSB-2 having high pathogenicity against first and second instars of predominant species Uttarakhand Himalayas, Anomala dimidia and Holotrichia seticollis [19]. However, its practical utility comes from potential performance under field conditions. With a view to ascertain its potential as augmentative bio-control, we conducted field trials to determine the relative effects of this bio-agent on the suppression of white grubs and its impact on other soil microbiota for ecological compatibility.
Materials and Methods
Field Evaluation of WGPSB-2

Site selection
Based on random survey on cropping pattern and white grub sampling in the mid hills (1000-1500 amsl) of Uttarakhand Himalayas five villages viz., Chausali, Naukot, Daulaghat, Govindpur and Manan (detailed in Table 1) in Almora district were selected for testing of WGPSB-2 in ‘adopted village’ concept. Dhaspad, a non-adopted village where no application of the bioagent, WGPSB-2 was done served as control. All the villages have an initial white grub population of one grub per 30x30x20 cm pit at all ten random locations covering the entire village. In each village, four individual fields (200 m²) were tagged for data collection.

Application of WGPSB-2
WGPSB-2 has a strong ability to colonize on different compost substrates. So, the application was planned in such a way that talc based formulation (1x10⁶ spores per gram) was initially applied to compost pits at a rate of 1kg/tonne for colonization [19]. After one month, WGPSB-2 enriched compost was evenly applied in the field during land preparation at the rate of 5 tons/hectare. The same procedure was followed in every year on community basis covering every field in entire village during first week of June, which coincides with the beetle emergence.

Data recording and statistical analysis
Pit sampling technique was used to monitor grub populations in the pre-tagged four fields in each village for four months from June to October for five years. During second week of each month, 30x30x20 cm pits were dug at three random locations and mean grub population of all the four fields per village was taken (grub population/month/village). The data obtained clearly showed that in any selected year, natural selection process directed the population density of white grubs to a seasonal decreasing trend from June to October. So, comparison of the mean grub population was made over the years in individual months as a base. The means were subjected to repeated measures ANOVA to compare grub reduction over the period, within the villages and interactive effects of village x time in SPSS software (version 10.0.1). The grub population densities of the adopted (Manan) and non-adopted village (Dhaspad) in July were also compared using two sample t-test.

Impact of WGPSB-2 on the soil microbiota
The experiment was conducted at ICAR-Vivekananda Parvatiya Krishi Anusandhana Sansthan (VKPAS), Experimental Farm, Hawalbagh (1250 amsl), in a Completely Randomized Design (CRD) in 1m³ galvanized sheet walled micro-pits with three doses of the bio-agent viz., 1x, 2x, 4x (x is the recommended dose) and a control (no application of the bio-agent) with four replications. The treatment dose was mixed with top 15cm soil in each pit and sampling was done at monthly intervals up to 24 months. All the samples were analyzed for total bacteria, pseudomonads, free living nitrogen fixers and total fungal counts by using a standard soil-plate dilution technique as described by Seeley et al. [14]. Appropriate dilutions of sample were plated on selective medium and incubated at 28°C for 24-48hrs for bacteria and 72-96hrs for fungal counts. The impact of WGPSB-2 was determined using principal component analysis (PCA) using XLSTAT software (XLSTAT 2010) to display the correlations and affinities between different doses for tested micro biota.

Results and Discussion
Field Evaluation of WGPSB-2
Soil bacteria such as Bacillus thuringiensis, Paenibacillus popilliae, P. lentimorbus, Micrococcus sp., Serratia entomophila, S. proteamaculans and Tersinia entomophaga etc [5, 7, 12] predisposes the subterranean white grubs to a large array of diseases. An entomopathogenic bacterium, B. cereus stain WGPSB-2 native to Uttarakhand Hills reported to have biocontrol potential against predominant white grubs of the region [19]. The tangible usage of any entomopathogen comes from its sustainable field management of targeted pest under given ecological conditions. In any selected year, particularly under Uttarakhand mid hills condition, the period between June to August was considered as peak activity period of white grubs. Bonferroni pair wise comparison of pit sampling data evidently showed a significant numerical reduction in grub population in all the adopted villages over years in only two months viz., June (1²/4⁴⁸ and 2⁶/4⁴⁸ years with mean difference of 1.156 (P=0.047) and 0.190 (P=0.044), respectively) and July (1²/2⁶, 1²/3⁴, 1²/4⁵ and 1²/5⁵ years with mean difference of 0.934 (P=0.028), 0.924 (P=0.021), 0.992 (P=0.009) and 0.963 (P=0.020), respectively). August month also showed numerical reduction in grub population but not significant. Despite of the tested five months, this significance reduction in only two months can be attributed to specific activity of WGPSB-2 against early instars [19] and reduced susceptibility of later instars [15] coupled with their movement into deep soil profiles for pupation which was beyond the sampling pit depth i.e. 20cm. This is more evident in September and October months with erratic variations over years (Figure 1). The maximum reduction of grub population within a year (Figure 1) and subsequent maintenance of stumpy populations shows the viability of WGPSB-2 as both a preventive and curative biological control agent as applications targeted against the first instars are essentially preventive [11]. This type of stage specific bioactivity is also pronounced in white grub pathogenic nematodes [7, 8, 9, 10, 11] and fungi [20, 22]. Moreover, the susceptibility of developmental stage and time taken to kill the host are key points in developing a control strategy [1]. Moreover, the annual and prophylactic applications (prior to incidence of grub) of WGPSB-2 have additive effects with residual population of previous year which is evidenced by maximum grub reduction during initial years. With respect to individual villages, maximum reduction of grub population was recorded in Manan (94.2%) and Chausali (89.3%) in June and July, respectively over five years period. Repeated measures ANOVA of grub density over years showed a significant interaction between time (years) (F₅,₄₁₈ = 26.47, 51.22 and 13.68), villages (F₁,₄₁₈ = 28.25, 68.64 and 19.74) and time X villages (F₁,₄ = 79.56, 199.27 and 94.28) in June, July and August. By considering all the villages, an average reduction of 85.2 and 84.4% reduction in grub population is achieved over a five years period. A representative grub population in Manan (Table 2) also showed a significant reduction of more than 85% by fifth year and variability between individual months of test years. These minor non significant fluctuations in grub density are also reported in other villages which can be attributed to differences in their topographic structures, biodiversity and proximity to the forest areas. A t-test (n=12) comparison (Table 3) of grub populations between Manan (adopted) and Dhaspad (non-adopted) also showed significant difference.
between them with an equilibrium in grub density in latter (Figure 2).

**Impact of WGPSB-2 on soil microbiota**

Besides pathogenicity, environmental competency and compatibility of the bio-agent were key factor for any potential microbial control agent against soil pests [6] in order to have sustained management. A classical microbiological numbering technique was used to analyse soil microbial perturbations induced in soil over a period of 24 months. The data on tested microorganisms in both inoculated and non-inoculated micropits over a period of two years was presented in Table 4. Introduction of WGPSB-2 resulted in a significant increase of bacterial and cultivable fungi populations from one and 6 month post-inoculation, respectively until the end of the experiment. However, only a slight increase is noticed in case of Pseudomonads populations, which became significant after 6 months. These results showed that variations of the numbers of viable micro-organisms due to the introduction of WGPSB-2 were less important than the “non intentional” variations due to the experimental conditions and also observed in the non-inoculated soil.

Table 1: Details of the villages selected for study

| Sl. No. | Village     | Altitude (m amsl) | Block          | Geographical identity |
|---------|-------------|-------------------|----------------|-----------------------|
| 1       | Chausali    | 1133              | Hawalbagh      | 29.592°N, 79.5997°E   |
| 2       | Tunakot     | 1065              | Tarikhet       | 29.5765°N, 79.4698° E  |
| 3       | Daulaghat   | 1285              | Hawalbagh      | 29.4806°N, 79.2611°E  |
| 4       | Govindpur   | 1310              | Hawalbagh      | 30.19°N, 78.04°E      |
| 5       | Manan       | 1350              | Takula         | 30.7726°N, 79.4953°E  |
| 6       | Dashpad     | 1370              | Dhauradevi     | 29.5988°N, 79.6579°E  |

Table 2: Grub population in tagged fields of the adopted village, Manan (1350 m amsl)

| Experimental fields | 1st Year | 2nd Year | 3rd Year | 4th Year | 5th Year |
|---------------------|----------|----------|----------|----------|----------|
| Field-1             |          |          |          |          |          |
| June                | 1.8±1    | 2.0±2    | 2.0±2    | 1.9±1    | 1.8±1    |
| July                | 1.2±0    | 1.2±0    | 1.1±1    | 1.0±0    | 1.0±0    |
| Aug                 | 1.2±0    | 1.2±0    | 0.8±0    | 0.8±0    | 0.8±0    |
| Field-2             |          |          |          |          |          |
| June                | 1.2±0    | 1.2±0    | 1.2±0    | 1.2±0    | 1.2±0    |
| July                | 1.2±0    | 1.2±0    | 1.2±0    | 1.2±0    | 1.2±0    |
| Aug                 | 1.2±0    | 1.2±0    | 1.2±0    | 1.2±0    | 1.2±0    |
| Field-3             |          |          |          |          |          |
| June                | 1.4±0    | 1.4±0    | 1.4±0    | 1.4±0    | 1.4±0    |
| July                | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    |
| Aug                 | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    |
| Field-4             |          |          |          |          |          |
| June                | 1.7±1    | 1.7±1    | 1.7±1    | 1.7±1    | 1.7±1    |
| July                | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    |
| Aug                 | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    | 1.0±0    |

Table 3: Comparison of mean grub populations in July between Dashpad (Non-adopted) and Manan (Adopted village)

| Village     | Mean grub population ±SE |
|-------------|---------------------------|
|             | 2007          | 2008          | 2009          | 2010          | 2011          |
| Dashpad     | 2.5±0.31 (1-4) | 2.9±0.23 (2-4) | 2.8±0.37 (1-4) | 2.8±0.24 (2-4) | 2.8±0.21 (2-4) |
| Manan       | 1.2±0.22 (0-2) | 0.2±0.11 (0-1) | 0.2±0.11 (0-1) | 0.17±0.11 (0-1) | 0.2±0.13 (0-1) |

Table 4a: Indigenous microbial population densities assessed using culture plating method in soil before inoculation of entomopathogenic bio-agent *Bacillus cereus* strain WGPSB-2

| Microorganisms | Pits initial microbial population densities |
|----------------|--------------------------------------------|
| Total Bacterial counts | 4.5 x10⁶ | 6.4 x10⁶ | 5.4 x10⁶ | 6.1 x10⁶ |
| Total Fluorescent Pseudomonads | 2.3 x10⁵ | 2.4 x10⁵ | 2.8 x10⁵ | 2.3 x10⁵ |
| Total Diazotrophic Bacteria | 1.0 x10⁵ | 1.0 x10⁵ | 1.2 x10⁵ | 1.0 x10⁵ |
| Total Fungal counts | 1.2 x10⁴ | 9.0 x10³ | 9.0 x10³ | 9.0 x10³ |

Figures in parentheses are range.
Table 4b: Indigenous microbial population densities assessed using culture plating method in soil after inoculation of entomopathogenic bio-agent Bacillus cereus strain WGPSB-2

| Doses             | 1<sup>st</sup> | 6<sup>th</sup> | 12<sup>th</sup> | 18<sup>th</sup> | 24<sup>th</sup> |
|-------------------|---------------|----------------|----------------|----------------|----------------|
|                   | Total bacterial population (x10<sup>6</sup> cfu/g of oven dry soil) |               |                |                |                |
| T1 (1x)           | 5.7           | 13.9           | 8.02           | 20.0           | 2.75           |
| T2 (2x)           | 6.1           | 37.8           | 4.69           | 22.5           | 1.25           |
| T3 (4x)           | 3.7           | 6.7            | 4.94           | 29.0           | 3.50           |
| T4 (control)      | 5.5           | 13.2           | 3.97           | 22.5           | 2.10           |
|                   | Total fluorescent Pseudomonads (x10<sup>5</sup> cfu/g of oven dry soil) |               |                |                |                |
| T1 (1x)           | 6.56          | 262            | 36.2           | 28.0           | 40.0           |
| T2 (2x)           | 5.24          | 188            | 51.5           | 23.0           | 27.5           |
| T3 (4x)           | 4.63          | 178            | 63.9           | 28.7           | 35.0           |
| T4 (control)      | 2.79          | 94             | 71.2           | 30.0           | 30.5           |
|                   | Total Diazotrophic Bacteria (10<sup>3</sup>cfu/g of oven dry soil) |               |                |                |                |
| T1 (1x)           | 1.3           | 4.5            | 25.2           | 2.8            | 2.5            |
| T2 (2x)           | 1.1           | 4.0            | 13.6           | 2.8            | 1.9            |
| T3 (4x)           | 1.4           | 4.4            | 25.3           | 3.8            | 4.4            |
| T4 (control)      | 1.4           | 4.4            | 29.8           | 3.4            | 3.5            |
|                   | Total Fungi (x10<sup>4</sup>cfu/g of oven dry soil) |               |                |                |                |
| T1 (1x)           | 7.0           | 20.8           | 7.55           | 27.0           | 40.0           |
| T2 (2x)           | 4.0           | 31.4           | 5.75           | 8.5            | 40.0           |
| T3 (4x)           | 3.0           | 25.9           | 8.54           | 6.8            | 45.5           |
| T4 (control)      | 8.0           | 19.7           | 7.29           | 13             | 20.0           |

*Pits were inoculated with 1x, 2x and 4x doses of WGPSB-

Fig 1: Grub population reduction of targeted villages in different months for the month of June, (b) For the month of July, (c) For the month of August
**Fig 2:** Grub populations in non adopted village, Dashpad
Fig 3: Multifactorial comparison of the field experiment using PCA. (a) Correlation between indigenous microbial population densities, (b) Correlation between four treatments and time interval. Parameter codes: TBC-Total bacterial population; TFP-Total fluorescent Pseudomonads; TDB-Total diazotrophic bacteria and TFC-Total fungal counts

Conclusions
The present investigation on white grub management through tale based formulation of a native isolate of entomopathogen, B. cereus strain WGPSB-2 has provided eco-friendly, cost effective and sustainable technology option. The high degree of management provided by the bio-agent may be attributed to the nativity of the pathogen to the region. However, its wide spread usage comes from its performance over the different agro climatic conditions and its pathogenicity against other species of white grubs.

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References
1. Ansari MA, Adhikari BN, Ali F, Moens M. Susceptibility of Hoplia philanthus (Coleoptera: Scarabaeidae) larvae and pupae to entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae). Biological Control. 2008;47:315-321.
2. Douglas Inglis G, Goettel MS, Butt TM, Strasser H. Use of Hyphomycetous fungi for managing insect pests. In: Fungi as Biocontrol Agents Wallingford: CAB International. 2001;23-69.
3. Grosch R, Scherwinski K, Lottmann J, Berg G. Fungal antagonists of the plant pathogen Rhizoctonia solani: selection, control efficacy and influence on the indigenous microbial community. Mycology Research. 2006;110:1464-1474.
4. Hochberg ME. The potential role of pathogens in biological control. Nature. 1989;337:262-265.
5. Johnson SN, Benefer CM, Frew A, Griffiths BS, Hartley SE, Karley AJ et al. New frontiers in belowground ecology for plant protection from root-feeding insects. Applied Soil Ecology. 2016;108:96-107.
6. Klein MG, Georgis R. Persistence of control of Japanese beetle (Coleoptera: Scarabaeidae) larvae with steinernematid and heterorhabditid nematodes. Journal of Economic Entomology. 1992;85:727-730.
7. Koneru SL, Salinas H, Flores GE, Hong RL. The bacterial community of entomophilic nematodes and host beetles. Molecular Ecology. 2016;25(10):2312-2324.
8. Koppenhofer AM, Fuzy EM. Effect of white grub developmental stage on susceptibility to Entomopathogenic Nematodes. Journal of Economic Entomology. 2004;97:1842-1849.
9. Lee DW, Choo HY, Kaya HK, Lee SM, Smitley DR, Shin HK, Park CG. Laboratory and field evaluation of Korean entomopathogenic nematode isolates against the oriental beetle Exomalix orientalis (Coleoptera: Scarabaeidae). Journal of Economic Entomology. 2002;95(5):918-26.
10. Mannion CM, McLane W, Klein MG, Moysenko J, Oliver JB, Cowan D. Management of Early-Instar Japanese Beetle (Coleoptera: Scarabaeidae) in Field-Grown Nursery Crops. Journal of Economic Entomology.
11. Power KT, Ruisheng AN, Grewal PS. Effectiveness of *Heterorhabditis bacteriophora* strain GPS11 applications targeted against different instars of the Japanese beetle, *Popillia japonica*. Biological Control. 2009;48:232-236.

12. Ruiu L, Satta A, Floris I. Emerging entomopathogenic bacteria for insect pest management. Bulletin of Insectology. 2013;66(2):181-186.

13. Scherwinsky K, Wolf A, Berg G. Assessing the risk of biological control agents on the indigenous microbial communities: *Serratia plymuthica* HRO-C48 and *Streptomyces* sp. HRO-71 as model bacteria. Biocontrol. 2007;52:87-112.

14. Seeley HW, Vemardem PJ, Lee JJ. Microbes in action, a laboratory manual of microbiology, fourth ed. W.H. Freeman and Co., New York, NY, 1995.

15. Selvakumar G, Mohan M, Sushil SN, Kundu S, Bhatt JC, Gupta HS. Characterization and phylogenetic analysis of an entomopathogenic *Bacillus cereus* strain WGPSB-2 (MTCC 7182) isolated from white grub, *Anomala dimidiata* (Coleoptera: Scarabaeidae). Biocontrol Science and Technology. 2007;17(5):525-535.

16. Selvakumar G, Sushil SN, Stanley J, Mohan M, Anudeep, Deepak Rai, Ramkewal, Bhatt JC et al. *Brevibacterium frigoritolerans* a novel entomopathogen of *Anomala dimidiata* and *Holotrichia longipennis* (Scarabaeidae: Coleoptera). Biocontrol Science and Technology. 2011;21(7):821-827.

17. Sharma G. Indian phytophagous Scarabs and their management: Present status and future strategy. Jodhpur, India. Agrobios. 2002, 216.

18. Singh MP, Mishra PN, Bisht RS. Nature and extent of damage of white grub *Lachnosterna longipennis* (*Holotrichia longipennis* Blanch.) under various farming situations of Uttarakhand hills. Indian Journal of Entomology. 2004;66:277-280.

19. Sushil SN, Mohan M, Selvakumar G, Bhatt JC, Gupta HS. Isolation and toxicity evaluation of bacterial entomopathogens against phytophagous white grubs (Coleoptera: Scarabaeidae) in Indian Himalayan hills. International Journal of Pest Management. 2008;54(4):301-307.

20. Thamarai CC, Thilagaraj WR, Nalini R. Field efficacy of formulations of microbial insecticide *Metarhizium anisopliae* (Hyphocreales: Clavicipitaceae) for the control of sugarcane white grub, *Holotrichia serrata* F (Coleoptera: Scarabidae). Journal of Biopesticides. 2011;4(2):186-189.

21. Wielkopolsan B, Obrepańska-Stepłowska A. Three-way interaction among plants, bacteria, and coleopteran insects. Planta. 2016;244(2):313-332.

22. Xiangqun N, Chunqin L, Xing L, Qinglei W, Guangjun W, Zehua Z. Laboratory evaluation of entomopathogenic fungi against the white grubs, *Holotrichia obilata* and *Anomala corpulenta* (Coleoptera: Scarabaeidae) from the field of peanut, *Arachis hypogaea*. Biocontrol Science and Technology. 2011;21(5):593-603.