https://doi.org/ 10.48047/AFJBS.6.5.2024. 11328-11343

African Journal of Biological Sciences

Evaluation of *Photorhabdus* Bacteria as a Potential Forthcoming Biocontrol Agent of White Grubs

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Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi: *10.48047/AFJBS.6.5.2024.11328-11343*

Abstract

In India, soyabean pays a significantly to the Indian edible oil pool. It contributes 25% to the total oil production in the country. In recent years consumption of soyabean meal is increasing day by day in India. The productivity of soyabean is mainly hampered by infestation of insects viz. aphids, jassids, white flies and white grubs. White grub is a major pest that is found in various regions of western Maharashtra, India. In nature larvae of white grubs are voracious feed any kind of roots or subterranean stems. Biopesticides are ecofriendly and effective against the population of grub. Several entomopathogens are disease causing organisms which inevitably infect to white grubs, that kill them or decrease their future generations. *Photorhabdus luminescens*, a symbiont of *Heterorhabditis indica*, have insecticidal activity towards insect pests by exerting an array of toxic effects. In the present investigation, efficacy of developed formulation of *Photorhabdus* bacteria isolated from was *Heterorhabditis indica* was studied. The field trial was conducted at farmer field with soyabean crop infested by white grubs. The mean population of white grubs recorded before the initiation of application was uniform ranging from 2.66 to 3.08 larvae per plant. After 7 days of treatment with formulation of *Photorhabdus*, 52.94 % larval population was reduced. The significant reduction in white grub larval population i.e. 83.34 % was noted at 45 days after treatment (DAT) over untreated control.

Introduction:

Soybean has notable place in world's oilseed cultivation scenario, due to its high production, profitability, and vital role in retaining soil fertility. It contributes 25% to the total oil production in the country (Hazra *et al*., 2020). In recent years consumption of soyabean meal is increasing day by day in India. The productivity of soyabean is mainly hampered by infestation of insect viz. aphids, jassids, white fly and white grubs. The crops cultivated in kharif season are potato, groundnut, sugarcane, maize, pearl millet, sorghum, cowpea, pigeon pea, soybean (Srivastava *et al*., 1971), kidney beans and ginger etc. The rainy season crops are damaged by white grubs (Sharma *et al*., 2019). In India, Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, and Kerala states, *Holotrichia serrata* causes destruction to the all crops. (Chandel *et al*., 2019) (Bhawane *et al*., 2012). White grub is a major pest that is found in various regions of western Maharashtra, India. (Theurkar *et al*., 2012). In nature, all white grubs are polyphagous (Sharma *et al*., 2019) feed on any kind of root or subterranean stem (Veeresh *et al*., 1988). The white grub larvae in their early stage of life consume organic matter of the soil for its growth. As the life stages passes to second and third instars mostly feed on roots of crops. The feeding activity of the third instar grubs is the main reason for the economic significance of chafers (Chandel *et al.,* 2015) . The larvae feed on fibrous roots of crops, which affect the growth of different crops. In particular, crops having a tap root system are affected more as compared to crops with an adventitious root system (Srivastava *et al*., 1971)

Due to the significant impact of this pest on the agriculture sector's economic growth, many countries have implemented various technology to minimize the damage caused. (Sharma *et al*., 2019). Biopesticide are ecofriendly and potent against the larval population. Several entomopathogens (Subbanna *et al*., 2020) are disease causing organisms which inevitably infect to white grubs, that kill them or decrease their future generations (Singh *et al*., 1991). The major entomopathogens that infect white grubs are nematodes, bacteria, viruses, and fungus. (Chandel *et al*., 2019). The entomopathogens exert insecticidal properties, toxins, enzymes and other substances for controlling grubs (Arora *et al.,* 2000). The entomopathogenic fungi and entomopathogenic nematodes have received more attention because of their potential for use in the management of white grubs than other microorganisms. However, EPNs have a better ability to control soil pests compared to insects that feed on foliage (Sharma *et al*., 2011). In India, *S. carpocapsae* (1966) was employed to control pests in crops viz. rice, sugarcane and apple (Hasan *et al*., 2009). The efficacy of entomopathogenic nematode in field is mainly affected by desiccation (Lello *et al*., 1996), temperature (Grewal *et al*., 1994, Wu *et al*., 2023),

ultraviolet radiations (Gaugler *et al*., 1992), application timing (Georgis *et al*., 2006). These are the major reason for limitation in use of entomopathogenic nematode as biocontrol agent.

In field condition, several microorganisms, including *Paenibacillus popilliae, M. anisopliae, B. bassiana,* and *B. brongniartii,* have been found to be harmful to white grubs. These microorganisms have shown great potential to reduce the white grub population. (Yadava *et al*., 2000). The entomopathogenic bacteria offers considerable potential as biopesticide (Kakade *et al*., 2020). The efficacy of *Paenibacillus sp* and *Serratia sp* have been studied against white grubs (Chandel *et al*., 2019). *Photorhabdus luminescens*, a symbiont of *Heterorhabditis indica*, is obtaining significant objective in the field of pest management (Uma *et al*., 2010). Similarly, *Photorhabdus luminescens* exerts insecticidal effects on harmful agricultural pest independent of its host (Mohan *et al*., 2003) (Razek *et al*., 2003). Several toxin complexes have been secreted in the growth medium of *P. luminescens*, including proteases, chitinases (Liu *et al*., 2019) (Kakade *et al*., 2023), lipases, and broad-spectrum antibiotics. These secreted compounds have been found to exhibit insecticidal activity (Forst *et al*., 1996) (Akhurst *et al*., 2006) (Rodou *et al*., 2010) in various studies.

P. luminescens kills its insect host by secreting toxins into the insect's haemocoel. Four pathogenicity islands were identified in genome of *Photorhabdus luminescens* coding for toxins. It is classified into four major groups: toxin complexes (Tcs), *Photorhabdus* insect related (Pir) toxin, makes caterpillars floppy (Mcf) toxin, type III secretion system (Ffrench-Constant *et al.*, 2007) (Yang *et al*., 2006)). Multiple pore-forming toxins are secreted by *P. luminescens* bacteria; these toxins penetrate plasma membrane of cells to exert their effects. These toxins can either generate perforating pores which serves to dissipate key electrochemical gradients or act as a puncturing mechanism to translocate a cytotoxic molecule into the cytoplasm (Roderer *et al*., 2019). Tripartite toxin complexes (Tc toxins) are protein complexes with high molecular weight that exhibit insecticidal properties. These complexes were initially discovered in the bacterium *P. luminescens* which is associated with nematodes (Liu *et al.*, 2019). The Tc complex, responsible for oral toxicity, and the Mcf toxin, which causes a decrease in insect body turgor and ultimately death, are two examples of the categorized toxins that can be found in pathogenic islands (Cabral *et al.*, 2004). The *P. luminescens* genome contains a wide range of genes responsible for coding of hemolysins, adhesions, lipases, proteases, chitinases, toxins, and antibiotic synthesis.

The *P. luminescens* bacteria are isolated from white grub larvae by infected indigenous entomopathogenic nematode, *Heterorhabditis indica*. This bacterium has been studied for its chitinase enzyme having insecticidal properties (Kakade *et al*., 2023). The chitinase enzyme is

responsible for degradation of chitin present in insect's cuticle and gut lining. Due to damage caused at peritrophic membrane of gut, it results in reduction in feeding of insects and subsequent death of pest. The efficacy of developed formulation of *P. luminescens* is being studied against white grub larvae at laboratory level. This developed formulation can used to control the soil insect pest – white grub larvae by soil application.

The purpose of current study was to evaluate the effectiveness of the developed formulation of *Photorhabdus* bacteria as a control strategy against the tested pests. For this purpose, field trial was undertaken at farmer field to check the bioefficacy of *Photorhabdus luminescens* against white grub larvae infested soybean crop.

Materials and Methods

Bacterial Formulation

The formulation of *Photorhabdus* bacteria ($1x10^8$ CFU/ml) was prepared by the same method published earlier (Kakade *et al*., 2023). The formulation was used to evaluate the efficiency of *Photorhabdus* bacteria for controlling the population of white grub larvae in soybean field.

Field Evaluation of developed formulation of *Photorhabdus*

The experiment was conducted at farmer field, Gulunche, Maharashtra, India during kharif season 2022. It comprised two treatments with spacings 45 x 5 cm^2 and soybean variety KDS-726 (Phule Sangam)(Warpe *et al*., 2022). The seed rate and dose of chemical fertilizer were used as per recommendation of Mahatma Phule Krishi Vidhyapeeth, Maharashtra. The seed treatment with Vasantdada Sugar Institute's Rhizophospho biofertilizers *(Rhizobium* and Phosphate solubilizing bacteria) @ 100 ml 10 kg-1 of seeds was done. The sowing was carried out by tractor and followed recommended package of practices i.e. fertilizer 50:75:45 kg NPK ha^{-1} and 5 tons of Farm Yard Manure (FYM) ha^{-1} except pest management.

The plant damaged by white grubs infestation was observed in 11 randomly selected spots in a $1m^2$ area of the treatments at 7, 15, 30 and 45 days after application of formulation of *Photorhabdus* (Pandey *et al*., 2023). The observation on growth characters and yield were recorded from randomly selected plants per plot. Statistical analysis of observations of larval mortality and growth parameters was subjected to t Test.

Results

Bioefficacy of formulation of *Photorhabdus* against white grub larvae

The field efficacy of formulation of *Photorhabdus* bacteria against white grub larvae is presented in Table no-1. The mean population of white grub recorded before the initiation of application was ranging from 2.66 to 3.08 larvae per m^2. The larval population per m^2 0.50 ±

0.52 was recorded after 45 days after treatment of *Photorhabdus* as compared to untreated plot (3.00 ± 0.02).

Table No.1- Bioefficacy of formulation of *Photorhabdus* bacteria on white grub population

Treatment	Precount	Percent Mortality			
		7 DAT	15 DAT	30 DAT	45 DAT
Drenching of *Photorhabdus* ($1x10^8$ CFU /ml)	2.66 ± 1.30	1.33 ± 0.49	1.17 ± 0.38	0.92 ± 0.29	0.50 ± 0.52
Control	3.08 ± 1.16	2.83 ± 0.57	3.16 ± 0.39	3.16 ± 0.39	3.00 ± 0.02
Percent Mortality Over to control	-	52.94	63.15	71.05	83.34
t-cal	0.86	6.51	16.24	17.23	16.58
t- stat	2.20				

α-0.05 level *DAT- Days after treatment

Effect of developed formulation of *Photorhabdus* on population of pest

After 7 days of treatment of formulation of *Photorhabdus,* it was revealed that the 52.94 % larval population was reduced as compared to untreated control. The significant reduction in white grub larval population i.e. 83.34 % was noticed at 45 days after treatment (DAT) over untreated control. It was observed that reduction in white grub population increases as the days of treatment increases.

Fig.1 - Effect of *Photorhabdus* formulation on white grub larval population

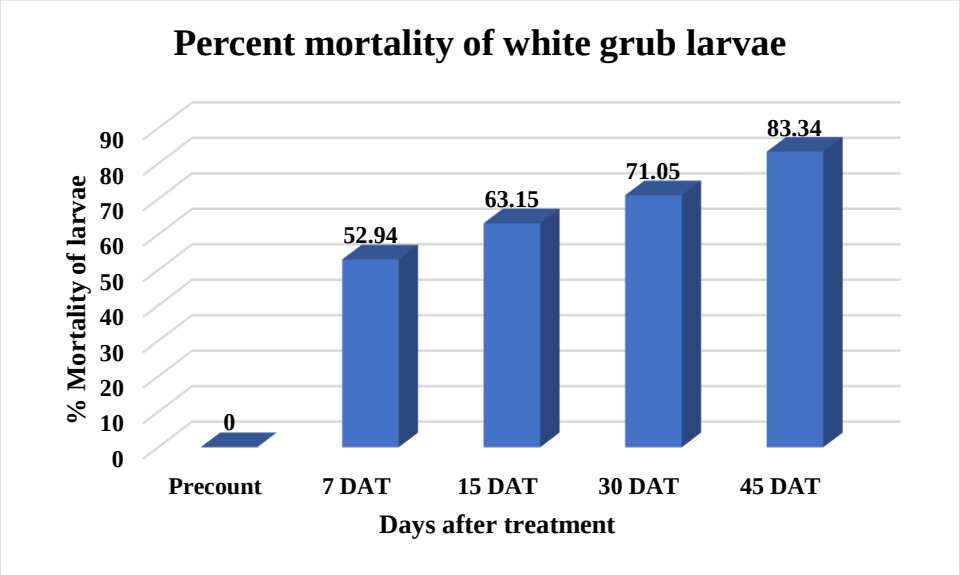
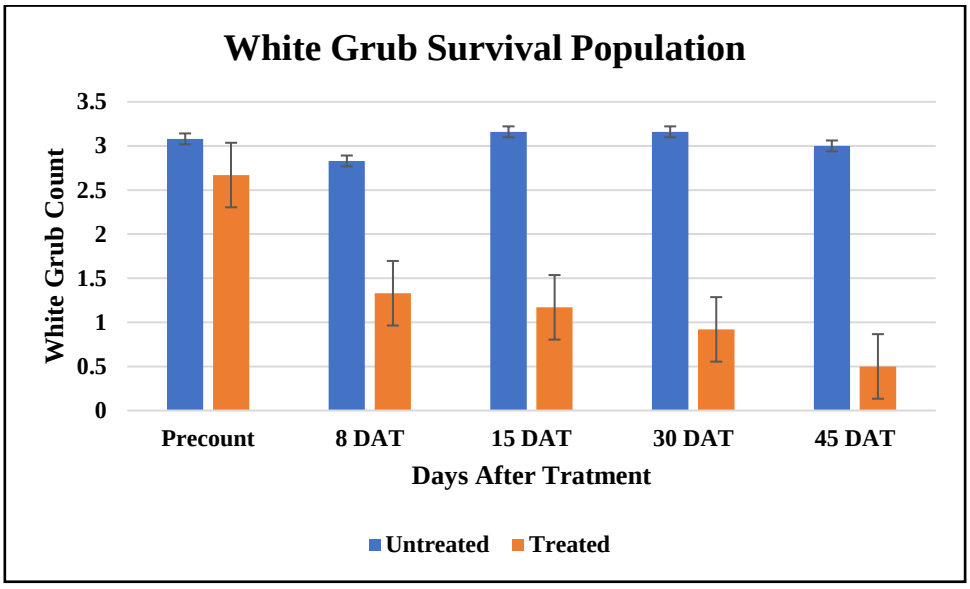


Fig. 2 –Survival population of white grub larvae after application of *Photorhabdus* formulation



The white grub survival population remained same in untreated plot whereas there was reduction in survival population in treated plot. The significant reduction in survival population of white grub larvae was observed after 7 days of treatment with highest reduction at 45 days after treatment as compared to untreated control. The significant reduction in white grub population was observed in treated plot as compared to untreated plot.

Crop growth characteristics in treated and untreated plot

The highest plant height was 51.50 cm recorded in treated plot as compared to untreated control 36.75 cm. Number of Branches Plant^{-1} was 7.40 observed as compared to untreated control (5.50). The number of pods plant^{-1} and number of seeds pod^{-1} was 94.49 and 2.78 over to control (80.75 & 2.32) respectively. The weight of pods plant^{-1} was 22.82 gms as compared to 18.90 gm in untreated control. The highest seed yield plant^{-1} was 11.91 gms as compared to untreated control (9.63gms).

Table No.2- t-Test to compare the effect of formulation of *Photorhabdus* bacteria on treated and untreated plot

Treatment	Plant Height (cm)	Number of Branches Plant^{-1}	Number of Pods Plant^{-1}	Number of Seeds Pod^{-1}	Weight of Pods Plant^{-1}(g)	Seed Yield Plant^{-1} (g)
Drenching of *Photorhabdus* (1x10^{8} CFU/ml)	51.50 ± 1.00	7.40 ± 0.54	94.49 ± 0.67	2.78 ± 0.19	22.82 ± 0.87	11.91 ± 0.55

Control	36.75 ± 3.18	5.50 ± 0.33	80.75 ± 0.36	2.32 ± 0.22	18.90 ± 0.44	9.63 ± 0.19
t-cal	13.44	11.25	73.58	4.49	14.01	13.26
t-stat	2.20					

α-0.05 level

Discussion:

In the present investigation, developed formulation of *P. luminescence* which was earlier isolated from haemolymph of white grub larvae infested by entomopathogenic nematode and evaluated its efficacy at field level against white grub larvae in soyabean crop. The percent mortality of white grub larvae was 52.94 % recorded at 7 DAT and it was increased as per days after treatment. The 83.34 % mortality of white grub larvae was recorded at 45 DAT. Similarly, the growth parameters; pod yield, seed yield, plant height were recorded significantly over to control. Many authors have reported the use of *P. luminescens* against Lepidoptera, Coleopteran, and Dictyoptera. (Bowen *et al*., 1998). The present research findings supported by the investigation carried out by Rajagopal *et al (*2002), 90 percent larval mortality of *Plutella xylostella* was reported after application of sprayable formulation of *P. luminescens* (10^6 CFU/ml) (Rajagopal *et al*., 2002). After 24 hrs of foliar application of *P. luminescens* IARI strain, 100 per cent mortality of cabbage butterfly was recorded (Mohan *et al*., 2003) (Uma *et al*., 2010). The results obtained by Mohan *et al*., were found parallel with the present investigation.

After treatment with the formulation of *P. luminescens,* mealy bugs became sluggish and ceased feeding within 12-24 hours. The colour of the dead mealy bugs changed from pinkish red to dark brown, which is a common sign of bacterial infection. The toxicity of *P. luminescens* affected both the nymphs and adults of the mealybug. The mortality rate gradually increased with time. (Fand *et al*., 2012). Earlier studies showed that the formulation of *P. luminescens* can be used as spraying or alginate beads to control pest infestation (Razek *et al.*, 2003) (Rajagopal *et al.*, 2002).

The secretion of bacterial toxin, both in primary and secondary forms, appears to have a significant impact on pest mortality. The bioassay results clearly demonstrated a correlation between cell concentrations and increased mortality, regardless of whether the bacteria were in their primary or secondary form. This occurs because of toxins secreted in high concentration by the bacteria. This explains that the insecticidal compounds produced by *P. luminescens* play a crucial role (Uma *et al*., 2010). Selcuk Hazir (2016) conducted a study on the effects of cell-free supernatants (10% v/v) derived from *Xenorhabdus* and *Photorhabdus*. The results revealed that, these supernatants exhibited inhibitory properties against the germination and growth of

various fungal and oomycete phytopathogens. The filtrate contains the metabolites that inhibit the growth of phytopathogens (Hazir *et al.*, 2004). The pest mortality occurs mainly by secretion of lipases, chitinases and proteinases (Bowen & Ensign *et al.*, 1998b) (Clarke *et al.*, 1995) by the bacterium. The enzymatic secretions directly act on the cuticular chitin of the insects, resulting in mortality.

According to Chen *et al* (1994) cell wall of fungal mycelial chitin gets degraded by *Xenorhabdus* and *Photorhabdus*. This finding explains the exo-chitinase activity of *Xenorhabdus* and *Photorhabdus* (Chen *et al*., 1994). In 1996 Chen *et al*., reported that exo-chitinase and endochitinase activity of *Xenorhabdus* and *Photorhabdus* protect the cadaver from fungal pathogens (Chen *et al*., 1996). Dominelli *et al* (2022) reported antifungal activity of *Photorhabdus* against *Fusarium* and demonstrated that chitin-degrading activity of *Photorhabdus* was associated by the chitin binding protein and the chitinase enzyme (Dominelli *et al*., 2022). *P. luminescens* establishes colonisation on the fungal hyphae, subsequently leading to the binding of chitin binding protein (CBP) to chitin present in the cell wall of phytopathogenic *F. graminearum*. This interaction facilitates degradation of fungal cell wall by chitinase, ultimately preventing infection of plants by the fungus. A chitinase gene from *X. nematophilus* has been discovered, which codes for a chitinase protein. This protein exhibits both endo and exo chitinase activities, making it quite exceptional. The chitinase exhibited toxicity towards the larvae of *H. armigera*, resulting in death. The chitinase protein demonstrated significant efficacy in suppressing larval growth. Significant changes in weight were noted in the surviving larvae that consumed a diet containing chitinase, and the rate of pupation was found low (Mahmood *et al.,* 2020). Thus, it was confirmed that insecticidal toxins secreted by *P. luminescens* have chitinase activity which plays significant role in biocontrol activity against insect pests.

Pathogenesis of *Photorhabdus* was studied in a *Manduca sexta* (Silva *et al*., 2002). In this investigation, the initial growth of bacteria was found in anterior part of midgut and later on its length. The bacteria can be located in the midgut epithelium's basal side and extracellular matrix folds. The *Photorhabdus* secretes array of toxins and secondary metabolites which destroy the midgut epithelium (Bode *et al.*, 2009) (Mathur *et al*., 2019). This ultimately results in slow feeding by the insect and leads to starvation. Upon death of insect, bacteria inhabit the tissue of insect and starts its bioconversion. *Photorhabdus* has garnered significant commercial interest as a promising biocontrol agent due to its remarkable ability to cause high levels of destruction to a diverse range of insects. Even a small number of *Photorhabdus* cells can effectively eliminate an insect larva (Ffrench-Constant *et al*., 2003), (Waterfield *et al*., 2002).

Furthermore, many research studies have confirmed the effectiveness of this bacterium as a potent insecticide. It can be applied directly to the leaves (Mohan 2003 *et al.*, Razek *et al.,* 2003) or in the form of alginate beads (Rajagopal *et al*.,2006).

Grubs have become more tolerant with every decade due to repeated application of conventional methods and practices, which is alarming since there isn't a single solution for this serious problem. We need to shift gears and tackle the issue from a different angle. The encouraging results of present investigation showing a lot of promise both on the ecological and economical front. The efficacy of *P. luminescens* as a biocontrol agent against white grub larvae under field condition was proved in the present investigation.

Conclusion:

White grubs are notorious for their destructive nature and have ability to feed on a wide range of plants. The current study concludes about the formulation of *P. luminescens* which is significantly effective against white grub larvae. The developed formulation of *P. luminescens* as a promising biocontrol agent substantially controls infestation of white grubs in soybean crop. This biocontrol agent plays a crucial role in enhancing sustainable agriculture by successful control of grubs as an alternative to chemical insecticides and its efficacy against other species of white grubs contributes to its widespread use.

Acknowledgement

The authors acknowledge the farmers for providing field to conduct the research trial and facilities provided by the Vasantdada Sugar Institute, Pune, Maharashtra, India to carry out the present research.

Conflict of interest

No conflict of interest.

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