Sustainable white grubs management through the use of indigenous entomopathogenic fungi *Metarhizium anisopliae*

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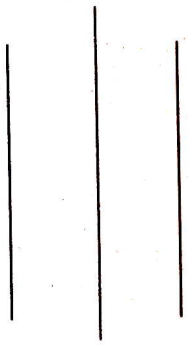

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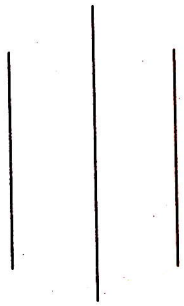

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Abstract

White grub (*Phyllophaga spp*) is economically most important and damaging insect pest of the agricultural crops like vegetables, maize and tea. The yield losses in ginger (80-100 %), in maize, vegetable and late harvested potato (20-50 %) and in newly planted tea (20%) in Bari land have been assessed. Chemical pesticides are recommended for the management of the insect but they are expensive and harmful to human, animals and environment health. The isolates of *Metarhizium anisopliae* found in the eastern hills are virulent and capable to kill the grubs. The formulation of the fungus in barley grains was found most suitable media. In cauliflower at Sindhuwa Dkankuta the white grub population decreased by 51% in fungus treated plot and yield increased by 17% (16 ton/ha). The results of verification /demonstrations revealed that in cabbage at Sindhuwa Dhankuta the white grub population was reduced by 16% in fungus applied plot and yield increased by 8% (52 ton/ha). In Ilam Panchkanya-7 the fungus applied in ginger crop reduced 50% population of white grub in the first year. Ginger yield increased by 9% (25.8 t/ha). In Panchthar Fidim-1 the fungus reduced white grub population by 65% in ginger and increased yield by 12% (30.9 t/ha). Similarly in Dhankuta Patle the white grub population by reduced by 55% in treated plots and yield was increased by 11% (31 t/ha).

Fungus formulation in barley grains need to be applied in furrow @ 40 kg/ha. Fungus kernel should be well covered by soil. Soil moisture is essential for the growth of fungus in the soil. The fungus application and its efficacy has some limitations.

Keyword

White grub, Metarhizium anisopliae, vegetable crops, isolates, virulent, bioassay

1. Introduction

White grubs (*Phyllophaga spp.*) are polyphagus in nature. It has become threat to agronomical and horticultural crops such as maize, millet, cauliflower, cabbage, tea, ginger, potato and other crops in the hills of Nepal. The lack of detail knowledge in species identification, bio-ecology and control management under Nepalese context has made very difficult to control white grubs. Farmers are also using indiscriminately various chemical pesticides at high doses (Information collected during field visit). Sindhuwa area of Dhankuta district (Potential area of commercial vegetable cultivation) also are using cypermethrin 10% EC up to 8 ml/litres of water for white grub control. Recently Sindhuwa Multipurpose Cooperative has started using granular form of Chloropyrifos (Dursban 10G) which is applied @ of 2kg per ropani for white grubs control which is quite higher than recommended dose and now facing problem for vegetable export to India. Thus there is urgent need of environment friendly and sustainable integrated management practices for the control of white grub, which this research project aims to find out. Thus, this project aims to make detail study on species identification, biology and management practices of white grub and manage white grubs using different biological agent such as *Metarhizum anisopliae* found indigenously.

The fungi *Metarhizum anisopliae* isolated from the eastern hills (Pakhribas) are more virulent than the isolates from Parbat district (tested in FAL communicated by SSMP). Moreover, bioassay of Pakhribas

isolates in laboratory at ARS, Pakhribas showed 71- 90% mortality of white grubs (Timsina, 2005). Therefore, these isolates could be more efficient than the isolates from other region for the management of white grubs in the eastern hills and can be used for the sustainable management of white grubs in the country as well, provided that the isolates are in their original virulence to the target insect. As women farmers are carrying out most of the farm activities including plant protection in hill areas. Therefore this project in long run aims to minimize the use of pesticides reducing environmental hazards and health hazards of women and children due to pesticides in the country.

More than 44% of the Nepalese farm families derive their livelihood from less than 0.5 ha of land (APP, 1995). Therefore for sustaining livelihood these farmers have intensified their land use and have initiated integrating high value cash crops such as potato, vegetables and ginger in their existing cropping system. As a result of intensification, soil insect pests such as white grubs and red ants have become threat to the sustainability of livelihood in the new intensified system. In hills of eastern Nepal the extent of damage by the grubs varies with the crop and season. In ginger, 80-100 % damage was reported where as in maize, vegetable and late harvested potato its damage ranged from 20-50 % and 20% in newly planted tea in Bari land (Timsina, 2003).

Farmers have limited alternatives of insecticides to control white grubs and other insects damaging their crops. However, they are not aware of the judicious use of pesticides. Maharjan *et al.* (2004) reported that farmers in the Koshi Highway corridor use different insecticides up to 22 times in a crop season. As vegetables is the third largest consumer of pesticides (1450g a.i./ ha) as compared to the national average of 142g a.i./ha (Thapa, 2003), the agro-business is in threat, as they do not have alternatives of the chemical pesticides for insect pest management during off-season cultivation. Some research carried out by ARS, Pakhribas and IAAS, Rampur indicated that there is an ample opportunity to explore isolates of *Metarhizium anisopliae* for the management of white grubs that can be an alternative to chemical pesticide.

The purpose of the project is to develop integrated white grub management techniques so as to increase the productivity of vegetable and cash crops and to contribute in livelihood improvement of eastern hill farmers. After the completion of the project at least one biological method for the effective control of white grubs adopted by participating farmers. The project has anticipated that through development and dissemination of biological control method will reduce the use of hazardous pesticides for the management of this pest. Moreover the farmers will be empowered with the knowledge of bio-agents that can kill the insect without harmful effect to the surrounding environment. This technology also contributes to the Integrated Pest Management programmed and organic crop cultivation which is a burning issue of the nation. The biological technology will contribute for the conservation of natural resources and biodiversity as well as in balancing ecology.

2. Materials and methods (conceptual framework, data, model, methodology) ?

This project was proposed to be accomplished by ARS, Pakhribas in close collaboration with Sindhuwa Multi-purpose Cooperative Limited Dhankuta, Mercy Corp Birtamod Jhapa and District Agriculture Development Offices of Ilam, Dhankuta and Regional Plant Protection Laboratory Biratnagar. The following activity were accomplished during the project period.

1.1 Base line survey to assess white grub severity and population dynamics in selected high value crops

Base line survey was conducted to find out the most white grub affected area, population dynamics of the species, severity of loss and management practices followed by the farmers. Based on survey data,

site for the field demonstration were selected

1.2 Collections of fungal pathogen from white grub cadaver and soil samples

Two hundred sample of grubs per location and five soil samples per plot were collected in order to get the large number of fungal pathogen associated with the white grubs. The collected grubs was reared in the laboratory at ARS Pakhribas. The cause of death was identified and recorded. The dead grub due to fungus *Metarhizium anisopliae* was be kept for the sporulation on the cadaver. The soil samples from the selected area was collected and fungus from the soil samples was also isolated using larvae of galleria as bait for fungus.

1.3 Preservation of collected fungus and maintenance of galleria population in laboratory

All the isolates were kept for long-term preservation for future reference. After each three transfer the fungus in media, the fungus was be included in the host passage to maintain its virulence intact.

The population of Greater wax moth *Galleria melonella* is necessary for the baiting of fungus in the soil samples. These insect larvae are also required for the monitoring of the fungus population in the fungus treated plots. This insects population was maintained in the laboratory by rearing them in the artificial diet as prescribed in the standard protocol.

3.4 Assess the core collection through bioassay.

The bioassay of the core collections was be carried out in larva of white grubs in controlled condition to identify the most virulent fungus pathogen that can be mass-produced. The bioassay experiment was conducted in the insect pathology laboratory following dipping method at 10^7 spore/ml of water. Total number of insect per treatment (isolate) were 90 for three replications i.e. 30 insect per replication and control (Deeping in distilled water) for each set of experiment.

The experiment was conducted in completely randomized design (CRD). Each insect was kept individually in 60 ml capacity transparent plastic bottles and fed with the slices of carrot. Weekly observation was taken for live and dead grubs and the food will be replace. The grubs that are use for the bioassay experiment was kept in quarantine for one month after collection from field to avoid the infection in the field itself.

2.1 Identification of suitable mass multiplication media

Possible fungus growing medium was selected. As the fungus needs nutrients for its development, maize grits, broken rice, barley grains and wheat grains were assessed for best fungal multiplication media/substrate.

The best isolates selected from the activity 1.4 was mass-produced in these medium. In this activity different method of fungus mass production was tested. The easiest, sustainable and locally accepted technique was developed.

The detail method for mass production was two stage production systems. The first stage is the blastospore production in liquid media, and second stage was inoculation of blastospores in solid substrates (barley grains, maize grits, broken rice etc). The composition of liquid media was followed as per Dr Keller protocol for mass production.

2.2 Identification of suitable fungal formulation

Suitable fungal formulation (granular, dust, etc) was prepared and their efficacy was tested at research

station. Granular formulation was prepared in barley grains, maize grits and broken rice were used for production of spore dust.

3.1 Handover Mass Production techniques

The local entrepreneurs such as cooperatives, pesticide sellers, farmer groups and government laboratories having capacity for the production of the fungus inoculums were identified and provided training on the mass production technique of quality inoculums. Mercy Corps at the time of project formulation ensured commitment for acquiring fund as a loan through Nirdhan Utthan Bank and other commercial bank for the local cooperative or private entrepreneur for the commercialization of the bio-pesticide particularly the *Metarhizium anisopliae*. Similarly Sindhuwa multi-purpose cooperative limited Dhankuta also showed interest and commitment for establishing laboratory for the commercial production and marketing of M. anisopliae. Sindhuwa multipurpose cooperative limited is marketing and exporting fresh vegetables in large scale.

3.2 Field demonstration

Farmers who are associated in groups in Mercy Corp Nepal and Sindhuwa Multi-purpose cooperative limited in general and poor and marginal farmers in particular, are practicing in intensified cropping system with the high value crops like off season vegetable, Potato and Ginger were selected for the experiment involving 75% female farmers. This activity was initiated using isolates already tested at ARS, Pakhribas from the first year of the project.

The plot size for each demonstration was 100 -150 m^2 depending up on the availability of land and farmers were considered as replication. At least five farmers in each site and two sites in each district were selected. After application of the fungus the population of grubs, infection rate and damage to crops were assessed and fungal population was monitored. Finally the crop yield was taken. The treated field and untreated field were observed by the collaborating organizations, farmers, reporters and other line agencies. The participating farmers played leading role to manage the experimental plots. Field visit and training were conducted at field level during cropping season with active participation of collaborators, team members, farmers, existing cooperatives and leader farmers.

3.3 Farmers training, stakeholder workshop, publication and distribution of booklets and posters.

Booklet was proposed to be published based on research outputs. Training and workshop were organized in consultation with the existing NGOs, DADOs. farmers' cooperatives, and RPPL. Logistic support for the participants was borne by the project.

3. Results

1.1Baseline survey to assess white grub severity and population dynamics selected high value crops:

A baseline survey was conducted in Dhankuta (Dhankuta Municipality and Parewadin V.D.C.), Ilam (Pashupatinagar, Godak and Panchakanya VDCs) and Panchthar (Fidim VDC) districts and identified the white grub population dynamics, white grub severe area, crop loss caused by the grubs and management practices followed by farmers.

The survey revealed that Crop farming is the major lively hood of the farmers which is followed by rearing livestock and vegetable farming. Cabbage farming is the major crop of the farmers of Parewadin VDC. However Potato, maize are the major crops of other research areas. During the past 5 years, there

is no significant change in cropping pattern. Farmers perceived that White grubs have become severe at different times in the past causing moderate to severe damage to agriculture. During the past 5 years, severity of White grubs increased in Cabbage, cauliflower, ginger and other crops. Crop loss of 5-80% has been occurred in different farmer's fields depending up on the crop.

Mixed cropping, crop rotation and use of chemical pesticides are the management techniques used by the farmers for the control of white grubs. Majority of farmers have not received any assistance from any government and non government agencies. Farmers ranked ginger as the first and cabbage as the second crop severely attacked by the white grub. Average number of grubs in 1m X1m plot was 2 which varied from 1 to 13.

The fungus Metarhizium anisopliae which has been found associated with insect collected and tested against the grub in the laboratory for their efficacy. The most virulent isolates are identified and maintained. These isolates will be used for the mass multiplication of for field demonstration. Furthermore these isolates are supplied to the private entrepreneur willing to produce in a commercial scale in local level.

1.2 Collection of fungal pathogen from white grub cadavar and soil samples: Two hundred grubs and soil samples (from each sites) were collected from different ecological zones (high, and mid hills of Dhankuta, Panchthar and Ilam). The collected grubs were reared in the laboratory individually in 60-100 ml capacity transparent cylindrical plastic bottles with cap perforated and observed for dead and alive on weekly basis up to three month. In order to maintain the fungal isolates and their virulence this activity is continuing. Soil samples (from each sites) are collected from different ecological zones (high, and mid hills of Dhankuta, Panchthar and Ilam) of the eastern Nepal in order to get the large number of fungal pathogen associated with the white grubs. Then the collected grubs are reared in the laboratory individually in 60-100 ml capacity transparent cylindrical plastic bottles with cap perforated and observed for dead and alive on weekly basis up to three month. The dead grub due to fungus *Metarhizium anisopliae* are kept on the surface of the soil on the rearing box and incubated at 27^0 C to allow for sporulation on the cadaver. The soil samples from the selected area are collected and fungus from the soil samples isolated using larvae of galleria (maintained in laboratory) as bait for fungus.

The fungal pathogens are isolated from the cadaver of the grubs and the cadaver of the galleria that inoculated in the soil samples in the selective and semi selective media. The media are prepared in petri plates and fungus spores inoculated with the help of platinum loop under aseptic condition. The inoculated plates are incubated at the temperature 25^0 C. After observing the spore colony in the media, the spores are transferred to the slants using single spore isolation so that other contamination in petri plates could be avoided and the pure culture of the fungus achieved.

1.3 Preservation of collected fungus and maintenance of galleria population in laboratory: All the isolates have been kept for long-term preservation for future reference. Fungus is stored in the refrigerator ($4\text{-}8^0$C) for up to one year. After each three transfers, a host passage has been repeated to maintain fungus virulence. This was done by infecting the original host of fungus i.e. white grubs, by the isolates of core collection.

The population of Greater wax moth *Galleria melonella* is necessary for the baiting of fungus in the soil samples. These insect larvae are also required for the monitoring of the fungus population in the fungus treated plots. This insects population has been maintained in the laboratory by rearing them in the artificial diet as prescribed in the standard protocol.

1.4 Assess the core collection through bioassay: The bioassay of the core collections has been carried out in the larva of white grubs in controlled condition to identify the most virulent fungus pathogen that can be mass-produced. The bioassay experiment was conducted in the insect pathology laboratory following

dipping method at 10^7 spore/ml concentration of spore suspension in water. Total number of insect per treatment (isolate) is 90 for three replication i.e. 30 insect per replication and control (Dipping in distilled water) for each set of experiment. The experiment was conducted in Complete Randomized Design (CRD). Weekly observation was taken for live and dead grubs and the food was replaced.

Grubs killed by the isolates differed significantly ($P<0.001$). Under control condition, grubs did not die due to fungal infection. Likewise, death of the grubs due to other factors did not differ significantly. Thus, the result indicated that the M. anisopliae isolates Pma 1 and Pma 2 are more virulent and more efficient in controlling white grubs among the tested isolates and can be used in mass production and field trials. Pma 1 killed the highest percentage (73.3%) of white grubs. However, Pma 2 was found statistically at par with pma 1.

2.1 Identification of suitable mass multiplication media: As the fungus needs nutrients for its development, Maize grits, broken rice, barley grains and wheat grains were assessed. The best isolates (Pma 1 and Pma 2) were selected from the activity 1.5 and mass-produced in these medium.

The detail method for mass production was two stage production system. First the blastospore production in liquid media and inoculation of blastospores in solid substrates (barley grains, maize grits, broken rice etc). The composition of liquid media was as prescribed by Dr Keller (Protocol for mass production).

Barley grains were the most suitable media for multiplication of M. anisopliae as fungal growth was better it in barley grains than other media. Other media has higher level of contamination problem. Moreover they took longer time for development of fungus.

2.2 Identification of suitable fungal formulation: As the fungus needs nutrients for its development, Maize grits, broken rice, barley grains and wheat grains were assessed. The composition of liquid media. Suitable fungal formulation (granular, dust, wettable powder etc) was prepared and their efficacy was tested at the research station. Granular formulation was prepared in barley grains, maize grits and broken rice will be use for production of spore dust. Blastospores of the fungus multiplied in liquid medium is inoculated in solid substrates (barley grains, maize grits, broken rice etc). Granular formulation in barley grains was the most suitable formulation for application of *M. anisopliae* in the field.

The experiment revealed that barley grains was the most suitable media for multiplication of *M. anisopliae* as fungal growth was better it in barley grains than other media. Other media has higher level of contamination problem. Moreover they took longer time for development of fungus. Activities like training to farmers, technicians and field demonstrations are being carried out for the dissemination of the technologies to the stakeholders

3.1 Handover Mass Production techniques:

In the process to hand over the technology the Training on the laboratory methods has been imparted to the members of SMCL, and officials of Regional plant protection laboratory Biratnagar, DADO Ilam and Dhankuta. The training on Metarhizium anisopliae, white grubs and use of the techniques has been given to farmers and extension technicians of the project areas.

Sindhuwa multi-purpose cooperative limited and Mercy Corps Nepal have not established laboratories for the production of the fungus product to date. They have not shown any initiatives in this regards because it requires a lot of investments and technical persons to handle the project. The plant protection officer of the RPPL has also been trained and they can take initiatives from public sector. ARS Pakhribas has doing its best to produce the fungus but in limited scale.

3.2 Field demonstration: Farmers associated in groups with Mercy Corp Nepal and Sindhuwa

Multipurpose cooperative limited and some other farmers were selected for the validation/ demonstration. This activity was initiated using isolates already tested at ARS, Pakhribas from the first year of the project. Field demonstrations on application and effectiveness evaluation of M. anisopliae were carried out in Dhankuta, Ilam and Panchthar districts on cabbage, cauliflower and ginger crops at field condition. Cabbage, cauliflower and ginger were the test crops for the validation /demonstrations in Dhankuta, Ilam and Panchthar districts where activities were initiated using isolates already tested at ARS, Pakhribas from the first year of the project.

Results on cabbage crop Demonstration : The cabbage variety Green Coronet was planted on 2069/3/10 and was harvested on 2069/10/9. In the Metarhizium anisopliae applied plots the average number of white grubs was recorded 6000/ha while in the control plot average number of white grub was 7000/ha. Damage due to white grub in the treated plot was 8000 plants/ha and in the control plot the damage was 12000 plants/ha. The yield of cabbage was obtained 52 ton/ha in the Metarhizium anisopliae applied plots which is 8.3% higher that the yield of control plot.

Field Demonstration on Cauliflower : The cauliflower variety white top was planted on 2069/3/15-20 and was harvested on 2069/6/8. The damage by white grub was found 5250 plants /ha in the Metarhizium anisopliae applied plots while in the control plots the damaged was as high as 10250 plants /ha. The yield of cauliflower was recorded 16.34 tons/ha in the Metarhizium treated plots while in the control plots the yield was 13.93 ton/ha. Due to the application of the fungus the cauliflower yield increased by 17%.

Field Demonstration on ginger: In Phidim of Panchthar district average number of white grubs per m^2 in treated plot was 0 but in non treated plot it was 0.6/m^2. Similarly, ginger weight was increased by 8% in treated plots in Phidim. White grub damaged ginger yield by 0.07% in *M. anisopliae* treated plots but 2.15% in non treated plots. In the Panchthar district ginger crop was planted on 2069/1/8 and was harvested on 2069/1/10-11. The local variety of ginger was planted. The number of white grubs in the treated plots was recorded 3500/ha which is 65% less compared to control plots. The ginger yield in the Metarhizium anisopliae applied plot increase by 12% compared to control plots.

The results of trial revealed that 12.41% higher ginger yield was obtained in *M. anisopliae* treated plots in Ilam. Weight of healthy rhizome was 25.41% higher but damaged rhizomes was 39.85% lower in treated plots than the untreated plots. Damage to the rhizome was significantly lower in treated plots (10.19%) than the untreated plots (22.45%) and 76.41% reduction in white grub population was observed in *M. anisopliae* treated plots.

In the Patle of Dhankuta district ginger crop was planted on 2069/1/26 and was harvested on 2069/ 9/23. The local variety of ginger was planted. The number of white grubs in the treated plots was recorded 2500/ha and in the control plots it was 5500/ha which is 45% less compared to control plots. The ginger yield in the Metarhizium anisopliae applied plot increase by 10.5% compared to control plots.
In Dhankuta, 7.47% higher yield was observed in treated plots (5.033 kg/m^2) as compared to non-treated plots (4.68 Kg/m^2). White grub damaged gingers and white grub numbers significantly reduced by 60% and 46% in treated plots than non treated plots.
In Panchkanya of Ilam district in ginger crop Metarhizium anisopliae applied plots gave 12.41% higher rhizome yield compared to untreated plots. Weight of healthy rhizome was 25.41 % higher. Damage by white grubs was significantly lower in treated plots (10.19%) compared to untreated plots (22.45%). Reduction in white grub population was 76.41% in Metarhizium anisopliae treated plots.

3.3 Farmers training, stakeholder workshop, publication and distribution of booklets and posters: Farmer training has also been conducted where 17 farmers participated. Regarding the publication a leaflet (500 copies) in Nepali language has been published and being distributed to farmer.

4. Discussion

The collection, isolation and bioassay of the *Metarhizium anisolpiae* fungus prevalent in the eastern hills of Nepal revealed that virulent isolates (pma-1 and pma-5) were present in nature and have capacity to kill the white grubs. The results indicated that this natural enemy of the insect is abundant in nature and there is scope and potentiality to isolate and prepare bio-pesticides for the effective and sustainable management of the white grubs without polluting the environment and endangering the human and animal health. The results of the demonstration on ginger, cauliflower and cabbage crops showed that application of the *Metarhizium anisopliae* product revealed effectiveness of the fungus on the reduction of the white grub larvae population up to 51% in cauliflower, 16% in cabbage in Dhankuta district. Similarly in ginger crop 50% in Ilam, 65% in Panchthar and 55% in Dhankuta districts. The crop yield was increased due to application of the fungus *Metarhizium anisopliae* in cabbage was 8%, cauliflower (17%), ginger (9-12%). These results are preliminary and as the time passes the growth of the fungus in the soil will multiply and thereby the fungus will action will also increase and will result in reduction of the insect population and ultimately increasing the yield. However the conditions of adequate moisture in the soil maintained and application of soil insecticides is avoided. The results obtained so far are encouraging and in agreement with the results obtained in other countries. Thus the promotion of this technology should be intensified. The NARDF should take initiative for the manufacturing, marketing and dissemination of *Metarhizium anisopliae* fungus through another project.

5. Conclusion and implication

- Fungus formulation in barley grains need to be applied in furrow @ 40 kg/ha
- Fungus kernel should be well covered by soil
- Soil moisture is essential for the growth of fungus in the soil
- The fungus application and its efficacy has some limitations
- In cauliflower at Sindhuwa Dkankuta the white grub population decreased by 51% in fungus treated plot and yield increased by 17% (16 ton/ha).
- In cabbage at Sindhuwa Dhankuta the white grub population was reduced by 16% in fungus applied plot and yield increased by 8% (52 ton/ha).
- In Ilam Panchkanya-7 the fungus applied in ginger crop reduced 50% population of white grub in the first year. Ginger yield increased by 9% (25.8 t/ha).
- In Panchthar Fidim-1 the fungus reduced white grub population by 65% in ginger and increased yield by 12% (30.9 t/ha)
- In Dhankuta Patle the white grub population by reduced by 55% in treated plots and yield was increased by 11% (31 t/ha).

6. Acknowledgement

The project coordinator and entire project team gratefully acknowledge the National Agricultural research and Development Fund (NARDF) Kathmandu for financially supporting the project. Thanks are also due to farmers associated with Sindhuwa multi-purpose cooperative limited Dhankuta, farmers of Patle Dhankuta, Farmers of Ilam and Panchthar districts for active participation in the field demonstration and survey works. The collaboration and cooperation of the district agriculture development offices and staff of Dhankuta,

Ilam and Panchthar is also highly appreciated. We would like to acknowledge regional plant protection laboratory Biratnagar who collaboration and interest shown in the project implementation. The help and support of staff of the NARDF is also duly acknowledged. The hard work and dedication of the technicians and field workers of ARS Pakhribas Plant protection laboratory is gratefully acknowledged.

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