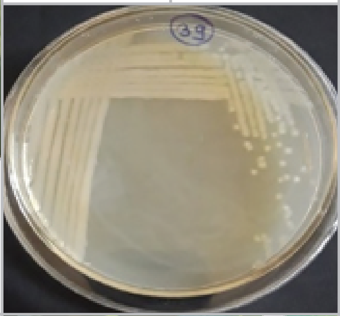
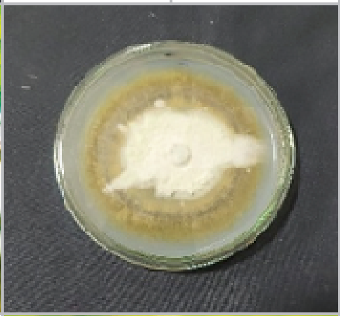
Bio-intensive Strategies for Disease and Insect Pest Management in Grapes





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ICAR-National Research Centre for Grapes, Pune

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Certificate of Acceptance of the final Report of the Project

We, **Dr.Kaushik Banerjee**, Director, National Research Centre for Grapes, Pune and **Dr. Narendra Pal Gangwar, Senior Manager - Product Development, International Panaacea Limited (IPL Biologicals), New Delhi** hereby certify that the final report of the project entitled **"Bio-intensive strategies for disease and insect pest management in Grapes: Case study Nashik, Maharashtra, India"** undertaken by ICAR-National Research Centre for Grapes, Pune-412307 as a collaborative project has been duly approved and accepted by both the organizations. It is agreed by both the parties that they will not accept any comment or suggestions that may arise in future on account of any review of the report by either of us or a third party.

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150 YEARS OF CELEBRATING THE MAHATMA

Bio-intensive Strategies for Disease and Insect Pest Management in Grapes

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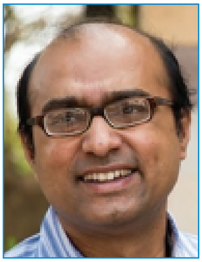

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1. Introduction

The grapevine is a significant fruit crop which is cultivated all over the world. In India, commercial grape production has seen substantial growth in recent years, particularly in regions like Maharashtra, Karnataka, Tamil Nadu, and Andhra Pradesh. The warm, humid and intermittently wet or cloudy weather in these regions is highly conducive for pest and disease attack on the grapevines. Diseases like downy mildew, powdery mildew, anthracnose and bacterial leaf blight, and insect pests like thrips, mealybugs, leafhoppers, flea beetle, red spider mites and caterpillars are the major challenging factors, management of which is necessary to get a productive, affordable and high-quality grape production. Many a times, these diseases and insect pests appear in the vineyards simultaneously, which causes reduction in yield and poor quality of grapes. To combat these problems in grape ecosystem farmers relies on pesticides applications as preventive and curative measures.

During past few decades it was observed that with the best use of chemical pesticides, many of the diseases and insect pests were not being controlled up to satisfactory levels in the major commercial belts of grapevine cultivation. The frequent use of pesticides i.e. 30-40 applications in a year, resulted in detection of their residues in/on grapes at harvest and these residues in turn affected the aroma, flavour, texture, or appearance of grape berries. Furthermore, there is reduction in nutritional values of grapes, and deterioration in the quality of the juice and wine. This causes huge economic losses to the grape farmers, due to the rejections in export as happened in the year 2003, in which Indian grape exporters catering to the European market received a pivotal wake-up call concerning the costs of failing to meet the EU's food safety standards. The grape consignments were rejected due to detection of the residues of the insecticides (e.g. methomyl, monocrotophos, etc.) in excess of the European Union (EU) maximum residue limits. About 75% of the samples exceeded the EU-MRLs. The European Union had rejected several grape consignments again in 2010 which incurred a huge financial loss to Indian farmers as they found excess levels of chlormequat chloride (CCC), a growth regulator, in grapes of India origin. According to 2019 FSSAI report, when market samples of grapes were tested, it was found that carbendazim fungicide residue exceeded the FSSAI MRL which is a concerning issue arising among the consumers. Moreover, their adverse effects on the environment and human health, coupled with the emergence of new pathogenic strains or biotypes due to pesticide resistance, have repeatedly highlighted their limitations. According to the Central Insecticide Board and Registration Committee, pesticides such as dichlorvos, dicofol, and methomyl were prohibited from use as of March 31, 2024. These chemicals are highly toxic and they persist in the environment for longer period which leads to soil and water contamination and their over-use results in development of resistance in pest population.

As farmers are concerned of these issues, the need for sustainable alternatives is more pressing than ever. Transitioning from conventional chemistry to green chemistry will put forward innovative solutions to reduce pesticide residues, minimum use of pesticide applications, pesticide resistance development and biodiversity conservation while maintaining agricultural productivity. Particularly, bio-intensive methods that refrain the chemical reliance, are gaining a lot of significance. These bio intensive methods include bio-control strategies, e.g., use of Entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* to manage the insect pests and use of fungi, *Trichoderma* spp. and Bacteria, *Bacillus subtilis* for the control of powdery mildew disease. And incorporation of these organisms i.e. Vesicular Arbuscular Mycorrhiza, Phosphate Solubilizing Bacteria, Potash Mobilizing Bacteria and Zinc Solubilizing Bacteria into soil will enhance the growth of the vineyard by supplementing nutrition. Therefore, these bio-intensive approaches which have been traditionally practiced for cultivating healthy crops and the ongoing challenges posed by pests and diseases have flagged so there is need to integrate these methods with modern plant protection technologies to enhance their efficacy.

A demonstration trial in Public-Private Partnership Mode was carried out on bio-intensive disease and insect pest management module by ICAR-National Research Centre for Grapes (NRCG), Pune, and IPL Biologicals Limited, Gurugram, Haryana (formerly known as International Panacea Ltd (IPL)) for three consecutive years 2019-20, 2020-21 and 2021-22 with the objective of producing pesticide residue free safe grapes. The model was based on minimum use of pesticides, induction of systemic resistance in vines, use of naturally occurring biological control agents, and conservation of natural enemies for production of residue compliant quality grapes.

2. Disease and insect pest challenges in grapes

2.1 Challenges in disease management

Disease management poses numerous problems in the major commercial grape belt in tropical regions of India, especially Maharashtra and some parts of Karnataka. Commercial white and coloured table grape cultivars are very vulnerable to downy mildew, powdery mildew, anthracnose and bacterial blight. Due to absence of cold induced dormancy, the grapevines are perennial and we follow "two pruning – one yield" system of grape cultivation wherein generally the foundation pruning done during April and fruit pruning is done during October. In these areas, susceptible green tissues are always present, unless shoot growth is manually controlled. A heavy load of inoculum in vineyard is observed during favourable warm, wet and humid weather conditions when prevailed during monsoon and post-monsoon season. Timely foliar spray management of disease are not possible due to intervention of factors, as the farmers are unable to manage disease effectively, they rely on self-defeating practices like increasing dosage or number of fungicide application. Furthermore, cost of cultivation will high and inadequate disease control results in resistance development. And food safety is major concern due to arising detection of fungicide residues at harvest.

2.1.1 Downy mildew

Downy mildew is one of the most important biotic constraints in grape cultivation which declines the production of the crop in regions viz., Maharashtra and adjoining areas of Karnataka. It is caused by the obligate fungus, Plasmopara viticola (Berk. & Curt.) Berl. & de Toni (Lafon and Clerjeau, 1988). The fungus infects the leaves, generating olive yellowish irregular spots on the upper surface and downy white growth on the below surface. The affected area becomes brown and brittle, dry and fall off. Infection happens through the stomata, which appear only on the lower surface of grape leaves and other green tissues. Mycelium proliferates intercellularly and penetrates cells through haustoria for obtaining nutrients. It reproduces asexually through sporangia produced on tree-like sporangiophores that protrude through the stomata. Sporangia germinate to produce biflagellate zoospores that swim to the stomatal openings, resulting in new infections. Hence, infection can only transpire in presence of free water on susceptible host tissues. Sexual reproduction generally occurs at the end of the season and results in the production of oospores which are also serves as survival structures. Oospores of *P. viticola* in infected leaf and cluster samples were not found when samples were observed across Maharashtra, which indicated that survival of P. viticola is in the form of sporangia.

Downy mildew can cause up to 100% crop loss under prolonged wet weather conditions that are ideal for sporulation and infection. During the monsoon season, leaf and stem infections have an impact on vineyard growth.

Downy mildew infection on lower surface

Pale yellow patch formation on upper side due to Downy mildew

2.1.2 Powdery mildew

Powdery mildew is caused by the obligate fungus *Erysiphe necator* Schw (previously known as *Uncinula necator* (Schw.) Burr). It appears in an epiphytotic form under favourable conditions. The disease is characterized by powdery patches on leaves, canes, tendrils, flowers, early fruits, and raches. The upper surface of leaves becomes dusty as powdery patches form, while the below surface shows mealy submerged growth. During dry conditions, affected leaves may curl upward. The fungus is an ectotroph i.e. the

Powdery Mildew symptom on leaves

Powdery mildew symptom on bunch of grapes mycelium remains on the surface of plant and forms multilobed structures, called as appressoria through which it penetrates the cuticle and cell wall to absorb food material from the epidermal cells. Asexual reproduction occurs by conidia that develop in chains on perpendicular conidiophores. Sexual reproduction occurs after fusion of two opposite mating types and giving rise to the formation of chasmothecia (cleistothecia). There are two bio-types of *E. necator* one develops chasmothecia and the other persists in buds and produces flag shoot symptoms after the bud sprouts. The latter bio type is not observed in vineyards in tropical regions of India.

During the summer, *E. necator* deposits conidia on grapevine bark. This observation is reinforced by the temperature endurance of conidia and the fact that as soon as favourable humid and cloudy conditions prevail, powdery mildew infections are found uniformly across the vineyard. powdery mildew infections can be found on vulnerable host tissues throughout year except during the hot summer months. Powdery mildew can form on clusters if humid and cloudy days prevail during flowering to fruit set period. The shelf life of infected bunches is shortened during storage due to fast desiccation.

2.1.3 Anthracnose

The disease is prevalent throughout the grape growing regions of the country. It is reported to be caused by the fungus, *Elsinoe ampelina*. However, our studies show the emergence of *Colletotrichum gloeosporioides* sensu lato as the dominant pathogen of anthracnose disease of grapes in India.

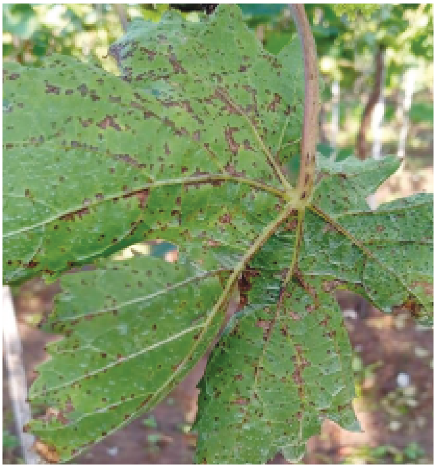
The disease is characterized by the lesions on shoots, leaves and berries. The first symptoms are small, yellowish spots on the leaves that eventually develop into circular, each spot turns greyish white in the centre and gets surrounded by a border of dark coloured zones. Numerous lesions are formed on the leaf and they may remain separate or get coalesced. Sometimes the central part of the spot falls out giving a shot hole appearance. And also, symptoms like cankers seen on stems and twigs if left untreated it will eventually kill the shoot.

The fungus found in the lesions/cankers in the shoot is the primary source of inoculum, which may be attached to the vines or found on the vineyard floor or in close proximity to the vineyards. *Colletotrichum gloeosporioides* is a necrotrophic fungus with a wide host range, therefore it can also survive on dead and decaying organic matter in the soil or on other hosts. When wet circumstances exist, conidia form and are disseminated by rain.

2.1.4 Bacterial Blight

Bacterial blight is caused by *Xanthomonas campestris* pv *viticola* which affects all aerial parts of the vine during wet and warm weather. It occurs on the vegetative growth during the monsoon, but it can even infect the forming bunches if rains are received in October-November. The first symptoms are tiny water soaked lesions on the lower surface of young leaves; these lesions grow larger, become cankerous, and angular. These lesions are mainly concentrated main and lateral veins. The bacterium perpetuates in the soil or crop debris as an inoculum and gets disseminated mostly by irrigation water.

Anthracnose shot hole symptoms



Bacterial leaf blight spots

2.2 Challenges in Pest Management

There are more than 132 insects that are reported to attack grapevine throughout the world. Nearly around hundred insect and mite pests have been reported to damage various parts of grapevine from different grape-growing states of India. Variations in climate, soil, varieties, rootstocks and distribution of natural enemies are the factors contributing for the distribution of grape pests. Pests like caterpillars, stem borers and grape flea beetles typically destroy the crop. Pests like leafhoppers, thrips and red spider mites suck the sap from the vine before the fruit is ripe. When these pests occur, the berries often become disfigured on the vine before ripening. Mealybugs infest the bunches, making them unfit for consumption. To have a balanced vineyard ecosystem, these pests need to be kept under check by their antagonists and natural enemies. As farmers over dependent on pesticides to manage these pests further erodes the natural balance and places them in a destructive cycle of repeated pesticide application. This leads to increase in cost of cultivation, resistance development, problems of pesticide residues in the final produce and environmental contamination. By avoiding broad spectrum chemical and going for need based safer pesticide application with use of biocontrol agents for production of residue compliant grapes.

2.2.1 Thrips

In peninsular India, thrips population is seen throughout the year. Thrips have now become a menace to control among other insect pests which cause damage in grapes because of their small size, short life cycle and ability to hide and get protection from feeding site under curled leaves. *Scirtothrips dorsalis* Hood and *Rhipiphorothrips cruentatus* Hood are regular pests of grapevines in peninsular India and are one of the most destructive pests of grapevine. Retithrips syriacus Mayet has also been observed in grapevines feeding on older leaves but this species was not found causing any economic damage so far. Both nymphs and adults cause damage by rasping the surface of the shoots, flowers and berries with their stylets and sucking the oozing cell sap. As a result of feeding on shoots, slow growth with stunted shoots and cupping of leaves were noticed. Thrips are known to cause scab formation on the berries. The affected berries develop a corky layer and turn to brown in colour. Thrips infested fruits are of poor quality and fetch low price in the market.

2.2.2 Leafhopper

Leafhoppers have become a major problem of concern because it requires large number of pesticidal application for managing their population which results in higher cost of cultivation and pesticide residues. *Amrasca biguttula biguttula* is a major pest in vineyards of Maharashtra. About 50 days after forward pruned grape vines are highly vulnerable to damage by these leaf hoppers. Both nymphs and adults cause damage by puncturing leaf cells of phloem and suck the sap from the leaves. The damage is first seen as a scattering of small white spots and later causes crinkling of the leaf, hardening of the leaf blade and browning of the nerves. With severe infestation and continuous sucking, the entire leaf turns yellow to brown. Later on, red spider mites and powdery mildew incidences are more in these curled leaves as they provide protection from pesticides as do not reach inside, the curled leaves.

Curling and cupping of leaves due to thrips feeding

Leaf curling due to leafhopper damage

2.2.3 Mealybug

Mealybug is one of the most destructive pests of vineyards and it is difficult to manage. Maconellicoccus hirsutus and Planococcus citri are the major infesting species of grapes in peninsular India. Both adult and nymphs are phloem feeders and suck the sap from tender vine parts during new emergence stage after fruit pruning which results in malformation of leaves and shoot tips. Mealybugs excrete honeydew that serves as a substrate for the growth of sooty mould which inhibits photosynthesis and affects growth and development of vine. Infested bunches develop shrivelled berries, and they do not fetch any market value. Association with ants will further aggravate the problem as they in migration of pest. Mealybugs have reported to cause total yield losses in the field.

Mealybug infested bunch

2.2.4 Flea beetle

Scelodonta strigicollis is a specific pest of grape vineyard. The adults are metallic brown in colour with black spots on elytra. Adult beetles are the damaging stage which cause damage to buds, tender shoots, tendrils, leaves and rarely the bunches. They bite the sprouting buds or eat them completely, and damaged buds fail to sprout and dry up. Damage symptoms are characterized by Linear and rectangular shaped holes on the leaves by this pest. These beetles avoid sunlight and remain hidden under shade. Flea beetle grubs are seen in the soil feeding on the roots but do not cause economical damage. They mainly feed on the new flush in October and April after pruning. Sometimes, management becomes challenging because foliar application is carried out to manage adults but as the larva are inside the soil, they are not controlled which results in adults population to build up.

Scelodonta strigicollis

Flea beetle damage on buds

2.2.5 Caterpillar

Spodoptera litura F. is the major lepidopteran species infesting grapevines. Sometimes, hornworn may be also noticed which causes defoliation during rainy season. The early instar of Spodoptera larvae skeletonizes leaves, and fully grown-up larvae feed on the whole leaves and cut the rachis of grape bunches and petioles of individual berry during night hours, thus leading to fruit drop, which causes significant economic damage. The caterpillars also feed on the unripe berries of pea size by making small scoops. The major damage to grapes is seen after forward pruning by Spodoptera in sprouting stage. The damaging stage is the larvae which are nocturnal and found hiding below small stones and debris on the ground during daytime.

2.2.6 Red Spider Mite

Tetranychus spp. is a serious pest causing damage to grapes in tropical India. The population to build up from second week of December and reaches peak in February-April. Both nymphs and adults prefer to suck the sap from older leaves but increase in population leads them to migrate even to bunches. Under heavy infestation, webbing is observed where mites remain inside the web and suck the sap. These causes leaf chlorosis which affects photosynthetic process which leads to reduction in sugar accumulation in berries. Excessive use of foliar application of imidacloprid, methomyl, lambda cyhalothrin, spinosad, etc., should be avoided after 50 days of pruning which kills natural enemies of mite. Many alternate host plants like Parthenium, etc. are the breeding grounds for mites

Spodoptera litura damage on leaves

Mite infestation symptoms on lower leaves

3. Methodology

Bio-formulations manufactured in ICAR-NRCG, Pune and IPL Biologicals Limited, Gurugram were used for conducting the trials and applied as preventive soil and foliar applications (Table1).

Table 1. Details of the bio-formulations

Sr. No.	Biologicals	Strain No.	Method of Application	Manufac-tured by
1.	*Trichoderma asperelloides*	5R	Soil through drip and foliar spray	ICAR-NRCG, Pune
2.	*Bacillus subtilis*	DR39	Foliar spray	
3.	*Ampelomyces quisqualis*	IPL/AQ/06	Foliar spray	IPL Bio-logicals Limited, Gurugram
4.	*Pseudomonas fluorescence*	IPL/PS/01	Foliar spray	
5.	*Beauveria bassiana*	IPL/BB/MI/01	Soil through drip and foliar spray	
6.	*Metarhizium anisopliae*	IPL/KC/44	Soil through drip and foliar spray	
7.	*Verticillium lecanii*	IPL/ VL/05	Soil through drip and foliar spray	
8.	Vesicular Arbuscular Mycorrhiza	IPL/VAM/01	Soil through drip	
9.	Potash Mobilizing Bacteria (PMB)	IPL/KMB/01	Soil through drip	
10.	Phosphate Solubilizing Bacteria (PSB)	IPL/PSB/01	Soil through drip	
11.	Zinc Solubilizing Bacteria (ZSB)	IPL/ZSB/01	Soil through drip	

For NRCG strains, in all bio-intensive plots, *T. asperelloides* 5R was applied twice before fruit pruning and three to four times after pruning. Only few systemic fungicides were used at high disease risk periods, based on weather and crop growth stage based disease predictions. IPL's biocontrol strains such as *Metarhizium anisopliae* (IPL/KC/44), *Beauveria bassiana* (IPL/BB/MI/01) and *Verticillium lecanii* (IPL/ VL/05) were applied as preventive (below ETL) to control mealybugs, thrips, mites, jassids and caterpillars and insecticide applications were need based.

Bacillus subtilis DR-39 was applied twice in the 30 days span before harvest period.

Water volume used for sprays was calculated based on the requirement i.e., 1000 L/ha at full canopy. Knapsack sprayer fitted with hollow cone nozzle was used for spray. Separate sprayer was maintained for chemical and bio-control agents. Pesticidal applications were minimal and need based (Table 2).

Table 2. Details of Fungicide and Insecticide sprays application

Sr. No.	Farmer name	Fungicide applications/ fruiting season						Insecticide applications/ fruiting season					
		2019-20		2020-21		2019-20		2020-21		2019-20		2020-21	
		BP	FP	BP	FP	BP	FP	BP	FP	BP	FP	BP	FP
1.	Kailash Pagar	6	15	4	10	4	12	6	10	4	12	3	13
2.	Vijay Dugje	5	14	6	12	4	14	6	12	5	14	5	12
3.	Suraj Vijay Vidhate	6	12	5	12	5	10	5	12	5	14	4	10
4.	Manoj More	4	12	4	14	3	11	6	11	6	12	5	10
5.	Sadanand Nathe	5	10	4	15	4	10	7	10	6	12	5	12

BP- Biointensive Plot FP- Farmers Plot

The biointensive module was implemented at five out-station fields of farmers at Nashik during grape fruiting seasons. This module was compared with the farmer practice (FP). The details of the biointensive plots (BI) are mentioned as follows:

Table 3. Farmers details

Sr. No.	Farmers Name	Location	Variety
1.	Kailash Pagar	Karsul	Clone 2A
2.	Vijay Dugje	Ahergaon	Sharad Seedless
3.	Suraj Vijay Vidhate	Ahergaon	Clone 2A
4.	Manoj More	Pimpalgaon	Sharad Seedless
5.	Sadanand Nathe	Antarveli	Clone 2A

This module was compared with the farmer practice (FP). One acre area for each treatment was allotted at every location. Observations on disease intensity, pest incidence, yield and residue were recorded.

4. Results

4.1 Disease Status

At all locations, the percent disease intensity for all diseases was less in bio-intensive plot as compared to FP. The initiation and development of diseases was delayed by 3-4 days in bio-intensive plot as compared to FP.

Among all diseases, the intensity of powdery mildew was higher at all locations during 2019-2020. Incidence of rust disease was absent in both the plots at Pagar, More and Nathe Farm. At Pagar, Dugje, Vidhate and Nathe farm, the intensity of bacterial leaf spot was lower as compared to FPs.

During 2020-2021, the intensity of downy mildew was more as compared to other diseases. The initiation of downy mildew, powdery mildew and anthracnose was delayed in bio-intensive plots as compared to FPs. At Dugje, Vidhate and Nathe farm the intensity of downy mildew and bacterial leaf spot was lower as compared to FP. At Pagar, Dugje and Nathe farm the intensity of powdery mildew was also less as compared to FP.

In 2021-2022, the percent disease intensity of downy mildew, powdery mildew and anthracnose was significantly lower in bio-intensive plots as compared to FPs at Pagar, Dugje and Vidhate Farm. Bacterial leaf spot and rust intensity were lower in bio-intensive plot at Nathe and More farm.

The study revealed that in all the three consecutive years the per cent disease infestation were less in bio intensive plots as compared to farmers plot and development of disease was slow in BPs. The NRCG pathogenic strains were used as preventive soil and foliar applications in as these strains of some bio-control agents which are effective in disease management and biodegradation of pesticides in grapes.

A strain of *Trichoderma afroharzianum* (NAIMCC-F- 01938) was found to able to reduce powdery mildew when applied as foliar spray with a count of 5 x 108 conidia /ml. Its hyphae grew towards and coiled around Erysiphe necator conidia, caused distortion of conidial structure and overgrew the powdery mildew colonies. Similarly, two strains of *T. asperelloides* i.e. strain 5R and strain NAIMCC-F- which when applied through the drip circle with a count of 5×10^8 conidia/ ml could reduce disease. Induced resistance is another broad-spectrum disease control method that is based on the activation of the plant's defence mechanisms is a promising low-impact approach for the control of crop diseases. All the identified strains induce the systemic resistance in grapevine.

Moreover, applications of *Bacillus subtilis* enhanced control of powdery mildew. Bio-augmentation of the grape fructosphere by *Bacillus subtilis* (strain DR-39) can enhance the degradation rate of multiclass fungicides and insecticides. Higher disease control is obtained with the intervention of biological strains. *Ampelomyces quisqualis* (IPL/AQ/06) a hyper parasite of powdery mildew fungi, grows fast and coils around the pathogen, penetrates through, thereby causing degradation of the cytoplasm and killing the pathogen. Hence, it acted as an effective biocontrol for powdery mildew pathogen as compared to farmer practice.

Similarly, *Pseudomonas fluorescence* (IPL/PS/01), which produces siderophore and chelates with iron and also secretes several plant growth substances like gibberellins compounds contribute to vigorous growth and inhibit the growth of disease causing pathogens. It is reported as an effective biocontrol agent against downy mildew, powdery mildew and anthracnose diseases. It combines very well with another bio-control agent such as Bacillus subtilis and enhances efficacies to control these diseases further.

Vesicular Arbuscular Mycorrhiza (IPL/VAM/01), Phosphate Solubilizing Bacteria (IPL/PSB/01), Potash Mobilizing Bacteria (IPL/KMB/01) and Zinc Solubilizing Bacteria (IPL/ZSB/01), which were applied in the soil, helped to supplement nutrition, enhanced growth and development of vines, disease control, better yield and quality of the produce.

4.2 Pest Status

During 2019-2020, damages due to thrips and mites were less in bio-intensive plot as compared to farmer practice at Dugje, More and Nathe Farm. At Vidhate Farm, damage due to thrips, mites and jassids were less in bio-intensive plot as compared to farmer practice. Damage levels for other pests were statistically at par with zero residue and FPs. Grapes from all the five plots were residue compliant at harvest.

Damages due to mealybug, jassids, caterpillar and mites were less in BP as compared to FP at four farms i.e. Vidhate, Nathe, Pagar and More Farm in the year 2020-21. Grapes from all the plots were residue compliant at harvest.

At More Farm, incidence of Mealybug and thrips caused less damage in BPs as compared to FPs during 2021-22. At Pagar Farm, damage due to caterpillars was less in BPs as compared to FPs. Thrips, mites, jassids and caterpillar damages in Dugaje Farm were less in bio-intensive plot as compared to FP.

The studies showed that the pest population of pests were less in bio intensive plots in comparison with farmers plot. IPL Biologicals strains Metarhizium anisopliae (IPL/KC/44) and Beauveria bassiana (IPL/BB/MI/01) which act by degrading the cuticle of the insects, penetrating the insect body, releasing toxins to kill insects and weakening the host's immune system, were used as stem wash and soil drench after pruning which helped to control mealybugs population significantly. Subsequently, plant wash with Metarhizium anisopliae (IPL/KC/44), Beauveria bassiana (IPL/BB/MI/01) and Verticillium lecanii (IPL/ VL/05) helped in managing the caterpillars and sucking pest's complex such as mealybug, thrips, mites and jassids.

4.3 Residue status

2019-2020:

At all locations, the pesticide detections in both the plots were below MRL but the residue content in bio-intensive plots were negligible as compared to FP. At Dugje farm, total six detections of pesticides were observed in FP and four in bio-intensive plot. At Pagar farm, three pesticide detections were recorded in both the plots but the residue content in bio-intensive plot was negligible. Six pesticide detections were observed in FP whereas three detections were observed in bio-intensive plot of More farm. At Nathe farm, ten pesticide detections were observed in FP whereas only five pesticide detections were observed in bio-intensive plot

2020-2021:

The number of pesticide residue detections was low in bio-intensive plots as compared to FPs. At Pagar farm, total four and three detections of pesticides were observed in FP and bio-intensive plot respectively. At Dugje farm, four detections were observed in both the plots but the residue content in bio-intensive plots was very low. At Vidhate farm, five pesticides residue were detected in FP and three detected in bio-intensive plot. At More and Nathe farm, six pesticide detections were observed in both FPs and two and three detections were observed in bio-intensive plots respectively.

2021-2022:

During this year, total four pesticides were detected in FPs and two fungicides were detected in BPs at Dugje farm. The detected residue content was below MRL in both the plots but in bio-intensive plot it was negligible with reduction in number of pesticide spray. At Vidhate farm, three pesticides were detected in FP and no detections were observed in bio-intensive plot. At Pagar farm, three detections of pesticides were observed in FP whereas, two detections in bio-intensive plot. At More farm, four detections at FP and no detections of pesticides in bio-intensive plot were observed. The number of pesticide detections were less in bio-intensive plot as compared to FPs.

Table 4. Number of detections from Farmer's Field

Sr. No.	Farmer Name	Number of detections					
		2019-20		2020-21		2021-22	
		BP	FP	BP	FP	BP	FP
1	Kailash Pagar	3	3	3	4	2	3
2	Vijay Dugje	4	6	4	4	2	4
3	Suraj Vijay Vidhate	5	7	3	5	-	3
4	Manoj More	3	6	2	6	-	4
5	Sadanand Nathe	5	10	3	6	2	3

Note: Detections for MRL level analysed at NRCG Lab.

These studies showed that the number of pesticide detection in all three consecutive years were less in bio - intensive plots where bio formulations were applied as preventive soil and foliar application in integration with minimum number of pesticide application, based on high disease and pest risk periods.

4.4 Yield & Economics

The three years yield data reflected the increase in yield of grapevine in bio-intensive plot at all locations. The increase in yield in the bio-intensive plot was higher during the year 2021-2022 than the year 2019-2020. During 2019-2020, the yield obtained from bio-intensive plot and FP of Pagar farm was 8.35 kg/vine and 5.17 kg/vine respectively. In the year 2021-2022, the yield was increased to 14.32 kg/vine and 9.69kg/vine respectively.

Similar results were obtained for other locations also. Yield levels notably enhanced in subsequent years.

Gross Income, Net Return and BC Ratio as worked out was significantly better in favour of BP compared to FP. From Pagar farm as an example, BC ratio improved significantly over the years in BP (1.13 in 2019-20, 3.07 in 2020-21 and 3.91 in 2021-22) as compared to FP primarily due to higher prices fetched in International Market owing to lower MRL levels and exportability potential. Net return and BC Ratio declined sharply in FPs due to sales of produce only in domestic market at a much lower price comparatively.

5. Suggestive Schedule of Bio-intensive Measures to Farmers

Pest & Disease Management in Grapes – October Pruning

Disease Management					
Spraying time	**Stage**	**Combination**	**Doses**	**Target**	**Remarks**
2 week before pruning (Stem Wash & Soil Appli.)	Pre- Pruning	*Bacillus subtilis* + *Trichoderma viride/harzianum/asperelloides*	5 ml each/L water	To reduce pathogen inoculum in vineyard soil and on plant	2 –stem wash spray, 2 soil Application (Total No. 4)
1 week before pruning (Stem Wash & Soil Appli.)	Pre- Pruning		5 ml each/L water		
4-5 days after pruning	On Dormant to swollen bud	*Bacillus subtilis* + *Pseudomonas fluorescence* (IPL/PS/01)	5ml each /L water	For ISR	Full coverage of plants
11-15 days after pruning	At three leaf stage		5ml each /L water	DM & PM	
16-35 days after pruning	At three leaf stage-		5ml each /L water	DM, PM & AN	
36-50 days after pruning	Flowering to fruit set		5ml each /L water	DM, PM & AN	

51-60 days after pruning	Berry Growth & Development	*Bacillus subtilis* + *Ampelomyces quisqualis* (IPL/AQ/06)	5ml each /L water	PM	
60-90 days after pruning	Berry Growth to veraison		5ml each /L water	PM/bunch rot	
90 days after pruning	Post Veraison up to Harvesting		5ml each /L water	PM/bunch rot	Helps in Bio-degradation of surface residues
	10 days before harvest-if diseses pm/bunch rot .				To control PM & Post harvest decay of pesticide

Note: DM- Downy mildew, ISR- Induced Systemic Resistance, PM- Powery mildew, AN- Anthracnose

Insect Management					
Spraying time	**Stage**	**Combination**	**Doses**	**Target**	**Remarks**
2 week before pruning (Plant Wash & Soil Application)	Pre- Pruning	*Metarhizium anisopliae* (IPL/KC/44) + *Beauveria bassiana* (IPL/BB/MI/01)	5ml each /L water (water volume 1.5 L/vine	To reduce initial stages of mealy bug population	Stem wash spray & Soil Drenching
1 week before pruning (Plant Wash & Soil Application)		*Metarhizium anisopliae* (IPL/KC/44) + *Beauveria bassiana* (IPL/BB/MI/01)	5ml each /L water (water volume 1.5 L/vine	To reduce initial stages of mealy bug population	Stem wash spray & Soil Drenching
1-2 days after pruning	On Dormant to swollen bud	*Metarhizium anisopliae* (IPL/KC/44) + *Beauveria bassiana* (IPL/BB/MI/01)	5ml each /L water (water volume 1.5 L/vine	Flea Beetle, caterpillar, ants - To protect new sprouting from chewing insects	Full coverage of plants (water volume 1.5 L/vine)
11-35 days after pruning	At five leaf stage	*Metarhizium anisopliae* (IPL/KC/44) + *Beauveria bassiana* (IPL/BB/MI/01)	5ml each /L water (water volume 1.5 L/vine	Mealy bugs, Thrips and Leaf Hoppers - Application when temp. is < 30 degree and RH is >60%	

36-50 days after pruning	Flowering to fruit set	*Beauveria bassiana* (IPL/BB/MI/01) + *Verticillium lecanii* (IPL/VL/05)	5ml each /L water (water volume 1.5 L/vine	Thrips, Leaf Hopper and mealy bug- At sightings	Full coverage of plants (water volume 1.5 L/vine)
50-75 days after pruning	Berry Development	*Beauveria bassiana* (IPL/BB/MI/01) + *Verticillium lecanii* (IPL/VL/05)	5ml each /L water (water volume 1.5 L/vine	Thrips, mites, Leaf Hopper and mealy bug- At sightings	
75-90 days after pruning	Veraison	*Beauveria bassiana* (IPL/BB/MI/01) + *Verticillium lecanii* (IPL/VL/05)	5ml each /L water (water volume 1.5 L/vine)	Mealybug & mites	
90 days after pruning	Veraison up to Harvesting before paper cover of bunches	*Beauveria bassiana* (IPL/BB/MI/01) + *Verticillium lecanii* (IPL/VL/05)	5ml each /L water (water volume 1.5 L/vine)	Mealy bugs, Thrips & mites	

Nutritional Schedule After October Pruning

Stages	Nutrition	Remarks
10 Days After Pruning	Vesicular Arbuscular Mycorrhiza (IPL/VAM/01) + Phosphate Solubilizing Bacteria (IPL/PSB/01) + Potash Mobilizing Bacteria (IPL/KMB/01)	Optimum Vine Growth
Early Shoot growth/ Pre Blooming Stage- 30-40 Days After Pruning	Zinc Solubilizing Bacteria (IPL/ZSB/01) + Phosphate Solubilizing Bacteria (IPL/PSB/01)	Good Early shoot growth and Blooming
Berry Growth Stage - 50-90 Days After Pruning	Vesicular Arbuscular Mycorrhiza (IPL/VAM/01) + Potash Mobilizing Bacteria (IPL/KMB/01)	Good Berry Growth and Development

Conclusion and Follow ups

- The use of a bio-intensive approach for managing diseases and pests have revealed that regular applications of bio-control agents reduce the incidences of disease and pests, even in condition where the environment are conducive to their occurrence or infestation.

- Over time, the population of pests and the disease inoculum are reduced by the regular use of biocontrol agents. This minimizes the need for pesticide applications to effectively control the disease and pests. Reduction in pesticides applications will have a direct influence on the input cost of growers. As a result of reduction in pesticide usage, detection of pesticide residues was minimized during harvest, which reduced the economic losses that grape growers suffered from export rejections.

- The use of biocontrol chemicals to grapevines resulted in the induction of systemic resistance, which enhanced the health of grapevine and increased the yield with exportable quality grapes

The microorganisms are being widely accepted in disease management approaches as they are environment friendly and they are touted as the novel means towards sustainability. These methods, although in place, need certain refinement and widespread application and awareness. However; their advantages are significant and have the potential to change the outlook on crop pest management.

The present study indicated that bio-intensive strategies comprising of need based application of chemical pesticides and predominant use of biocontrol agents, not only reduced the intensity of biotic stresses, but also resulted in a smaller number of pesticide residue detection, thereby fulfilling the goal of ‘Safe food for all’.

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16 MAR 2020

06 MAR 2020

प्रधान मुद्रांक विक्रीकर कोषागार पुणे करिता

Memorandum of Understanding

This Memorandum of Understanding (hereinafter referred to as the "MOU") is entered into on this 18 day of MARCH, 2020.

BETWEEN

ICAR-National Research Centre for Grapes (hereinafter referred to as **NRCG**) is a unit of 'The Indian Council of Agricultural Research' (hereinafter referred to as **ICAR**), registered under the Societies Registration Act, 1860 having its registered office at Rajendra Prasad Road, Krishi Bhawan, New Delhi-110001, being hereinafter referred to as "Party of the FIRST PART".

निदेशक / Director
भाकृअनुप–राष्ट्रीय अंगूर अनुसंधान केंद्र, पुणे 412307
ICAR-National Research Centre for Grapes, Pune-412307

Parties shall perform all their respective obligations for the projects that are underway.

7. Arbitration

Any dispute arising during operation of the MOU shall be settled through mutual consultations and agreement or arbitration and the arbitration proceeding shall be conducted in accordance with the provisions of the Arbitration and Conciliation Act, 1996, (as amended upto date) India. The venue of arbitration shall be at New Delhi, India.

8. Governing Law

This MOU shall be governed by laws of India and Courts of Delhi shall have exclusive jurisdiction to the exclusion of all other courts.

निदेशक / Director
भाकृअनुप-राष्ट्रीय अंगूर अनुसंधान केंद्र, पुणे 412307
ICAR-National Research Centre for Grapes, Pune-412307

Signature	Signature
(R. G. Somkuwar)	(Rahul Jatkar)
Director, ICAR-National Research Centre for Grapes, Pune	State Head-MH, International Panaacea Limited, Fursungi, Pune
Date:	Date:

Witness 1:

Witness 2:
ANIL CHITNIS
ZM - IPL / Pune.

Handing over Report