International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 544-550
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Evaluation of an Integrated Disease Management Module for Pearl Millet Against *Magnaporthe grisea*: A Causal Agent of Blast Disease

G.L. Basavaraj and K.N. Amruthesh*
Applied Plant Pathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysuru-570 006, Karnataka, India

Received: 10 Jan 2019 / Accepted: 9 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: dr.knamruthesh@botany.uni-mysore.ac.in

Abstract
The present-day disease management strategies are accomplished through various methods using a diverse source of pesticides which are hazardous to the whole environment and also have their limitations. In the present study, integrated disease management module with elicitors [*Pseudomonas fluorescens* (biotic) and BABA (abiotic)] and also tricyclazole (fungicide) individually and in combinations were evaluated for their effectiveness in growth promotion and disease protection against blast disease of pearl millet. It was noted that all the treatments significantly enhanced the plant growth parameters compared to control. There was an increase of 0.2 to 0.6 fold increase in all the plant growth parameters in elicitor-treated pearl millet seedlings individually and combinations. The results showed that the combination of *P. fluorescens* and BABA were more effective in disease protection of 69.75% and 67.25% under greenhouse and field condition, respectively which is in line with the growth parameter studies. The resistant seedlings offered 92.5% and 89.75% disease protection in greenhouse and field conditions. The results affirm the selected elicitors possess the potential to induce resistance in pearl millet against blast disease both under greenhouse and field conditions apart from enhancing the plant growth parameters.

Keywords
Pseudomonas fluorescens; BABA; pearl millet; *M. grisea*; plant growth

*****

INTRODUCTION
Disease management should move hand in hand with the present day climatic and environmental circumstances. The use of biological agents in disease management strategies are either not effective or sometimes fail to perform on embattled pathogens which have paved the way for finding alternate management methods [1]. Therefore, there is always

an endless search for the suitable amalgamation of different agents which not only reduce disease incidence and yield loss but also leads to sustainable agriculture. Integrated disease management (IDM) is one of the possible alternative methods for sustainable agriculture in an eco-friendly way. IDM is the combined application of different components like pesticides, bio-agents, cultural practices, host resistance, weather forecasting, soil amendment, etc., in an economically viable and ecologically safe proportion to achieve maximum efficiency in the management of the plant diseases [2].

In the recent past, many studies have been conducted in the management of plant diseases using IDM strategies [3-5]. It has been observed that *T. viride* (Tv3235) along with carbendazim (0.1%) and soil applications of FYM (1%) with sawdust (1%) offered maximum reduction in sheath blight severity, percent disease incidence and a substantial increase in grain yields over control in rice plants [6]. They also noted that the combined applications of bio-agent with chemical fungicides are an essential IDM package against sheath blight disease. Similarly, use of bioagents with soil organic amendments such as FYM, wheat straw, *Sesbania aculeata* (daincha), sawdust and neem cake worked effectively in the management of rice sheath blight disease and also increased the grain yield [7]. Further, researchers have also implied IDM using the combined treatment of biotic and abiotic inducers in the management of diseases in vast crop plants thereby indicating the effectiveness of the treatment [8].

Pearl millet (*Pennisetum glaucum* L.) is an important millet crop grown for its nutritious grains and stover and used in millions of households of tropical Asia and Africa. Pearl millet is a tropical cereal grown in areas with low rainfall and less soil fertility [9]. Its nutraceutical value and health benefits have made this cereal an emerging and a new age food among all social groups including urban and semi-urban communities. The yield and quality of pearl millet are affected by many diseases caused by fungal and bacterial pathogens. The blast disease of pearl millet caused by *Magnaporthe grisea* is one of the severe diseases which substantially reduce the crop yield if infected [10]. Several methods of disease protection have been employed in the recent past which includes the use of chemical pesticides, biological control, plant extracts, etc., and some results indicate a satisfactory level of disease protection but have their limitations [11-13]. Thus, an attempt has been made to assess the effect of IDM modules with fungicides, bio-agents and synthetic elicitors on the disease incidence of blast and growth parameters in pearl millet with comparison over the control under the greenhouse and field conditions.

MATERIALS AND METHODS

Collection of seed material

Pearl millet seeds ICMB95444 (susceptible) and ICMR06222 (resistant) cultivars were obtained from All India Coordinated Pearl Millet Improvement Project, Mysore Center, Mysuru, Karnataka, India and used throughout the study.

Elicitors

Pseudomonas fluorescens and BABA (50 mM) were used throughout the studies as they offered better protection and also enhanced plant growth parameters in pearl millet against blast disease caused by *M. grisea* (unpublished data).

Seed treatments

Pearl millet seeds (both susceptible and resistant) were surface sterilized with 0.02% sodium hypochlorite for 2 min and repeatedly rinsed with SDW (2-3 times). The surface sterilized seeds were treated with different treatments (Table 1) for 24 h and used throughout the study.

Table 1: Details of treatment modules for IDM of Pearl millet blast

Treatment No.	Preparation
T1	Susceptible seeds were soaked in sterile distilled water amended with CMC (0.2% w/v)
T2	Susceptible seeds were soaked in the suspension of *P. fluorescens* (1×10^8 CFU mL^{-1}) amended with CMC (0.2% w/v)
T3	Susceptible seeds were soaked in 50 mM BABA amended with CMC (0.2% w/v)
T4	Susceptible seeds were soaked in the suspension of *P. fluorescens* (1×10^8 CFU mL^{-1}) + BABA (50 mM) amended with CMC (0.2% w/v)
T5	Susceptible seeds were soaked in sterile distilled water containing Tricyclazole (0.2% w/v) amended with CMC (0.2% w/v)
T6	Susceptible seeds were soaked in BABA (50 mM) + Tricyclazole (0.2% w/v) amended with CMC (0.2% w/v)
T7	Resistant seeds were soaked in sterile distilled water amended with CMC (0.2% w/v)

Evaluation of IDM module for vegetative growth parameters of pearl millet under greenhouse and field conditions

The pearl millet seeds with different treatments (T1 to T7) were sown in earthen pots (9 × 9 cm diameter) containing autoclaved potting medium (1:2:1 sand, soil and farmyard manure) and maintained under greenhouse conditions (25 ± 2 °C). For field studies, trials were conducted at the experimental field of ICAR-AICPMIP, Mysuru center, University of Mysuru, Karnataka during Kharif (2017 and 2018). The plants were raised by following regular agronomical practices. After 60-days of sowing (DAS), vegetative and reproductive plant growth parameters *viz*., plant height, days required for 50% flowering, number of basal tillers, length and girth of ear head and 1000 seed weight were recorded accordingly. Each treatment consisted of four replicates.

Evaluation of IDM module for blast disease protection in pearl millet under greenhouse and field conditions

The pearl millet seeds with different treatments (T1 to T7) were sown in earthen pots (9 × 9 cm diameter) containing autoclaved potting medium (1:2:1 sand, soil and farmyard manure) and maintained under greenhouse conditions (25 ± 2 °C). For field studies, trials were conducted at the experimental field of ICAR-AICPMIP, Mysuru center, University of Mysuru, Karnataka during Kharif (2017 and 2018). The 14-day-old seedlings (both under greenhouse and field plants) were challenge inoculated with *M. grisea* (1×10^5 conidia mL^{-1}). Each treatment consisted of 10 pots, with ten seedlings per pot and the experiment was repeated thrice for greenhouse studies. For field studies, four replications per treatment consisting of four rows of 35 plants each were maintained in a complete randomized block design (6 × 6 m plots). Each row was 5 m long and 75 cm apart (between rows) with 15 cm spacing between plants within rows. The challenge inoculated plants were observed daily for the typical symptoms of blast disease like specks or spots on leaves which gradually enlarge to form spindle-shaped spots with necrotic greyish centres. Two readings of disease incidence were taken at 45 DAS and 60 DAS, respectively and disease protection was calculated using the following formula:

$$\text{Disease Protection (\%)} = \frac{\text{Number of infected plants}}{\text{Total Number of plants examined}} \times 100$$

Statistical Analysis

The mean data of all the experiments were subjected to analysis of variance (ANOVA) using SPSS Inc.16.0. Significant effects of treatments were determined by the magnitude of the F value ($p \leq 0.05$). Treatment means were separated by Tukey's HSD test.

RESULTS

Evaluation of IDM module for vegetative growth parameters of pearl millet under greenhouse and field conditions

The seed treatments with biotic and abiotic elicitors were able to enhance plant growth parameters compared to control plants. The results of the study specify that the selected elicitors didn't affect the plant growth parameters irrespective of conditions studied. It was noted that there was no significant difference in the fold increase among the elicitor treatments under both greenhouse and field studies. The pearl millet seeds treated with the *P. fluorescens* showed a plant height of 105.4 cm and 109.8 cm and 1000 seed weight of 10.9 g, while the seeds treated with BABA showed a plant height of 116.22 cm and 118.4 cm with 1000 seed weight of 10.83 g and 11.22 g under both greenhouse and field conditions, respectively. Seed treatment with both *P. fluorescens* and BABA enhanced plant growth parameters significantly compared to individual treatments under both greenhouse and field conditions (Fig. 1). The tricyclazole (0.2% w/v) treated seeds showed 120.25 cm and 128.24 cm of plant height and 11.82 g and 12.08 g per 1000 seeds, while the formulation of tricyclazole and BABA showed 125.62 cm and 130.9 cm of plant height and 12.18 g and 12.46 g per 1000 seeds at greenhouse and field conditions, respectively. There was an increase of 0.2 to 0.6 folds in seeds treated with selected elicitors (individually and in combination), while 0.2 to 0.7-fold increase was observed with the formulation treatment of tricyclazole compared to control seedling under both greenhouse and field conditions. The control resistant seeds offered a maximum improvement in plant growth parameters compared to all other treatments.

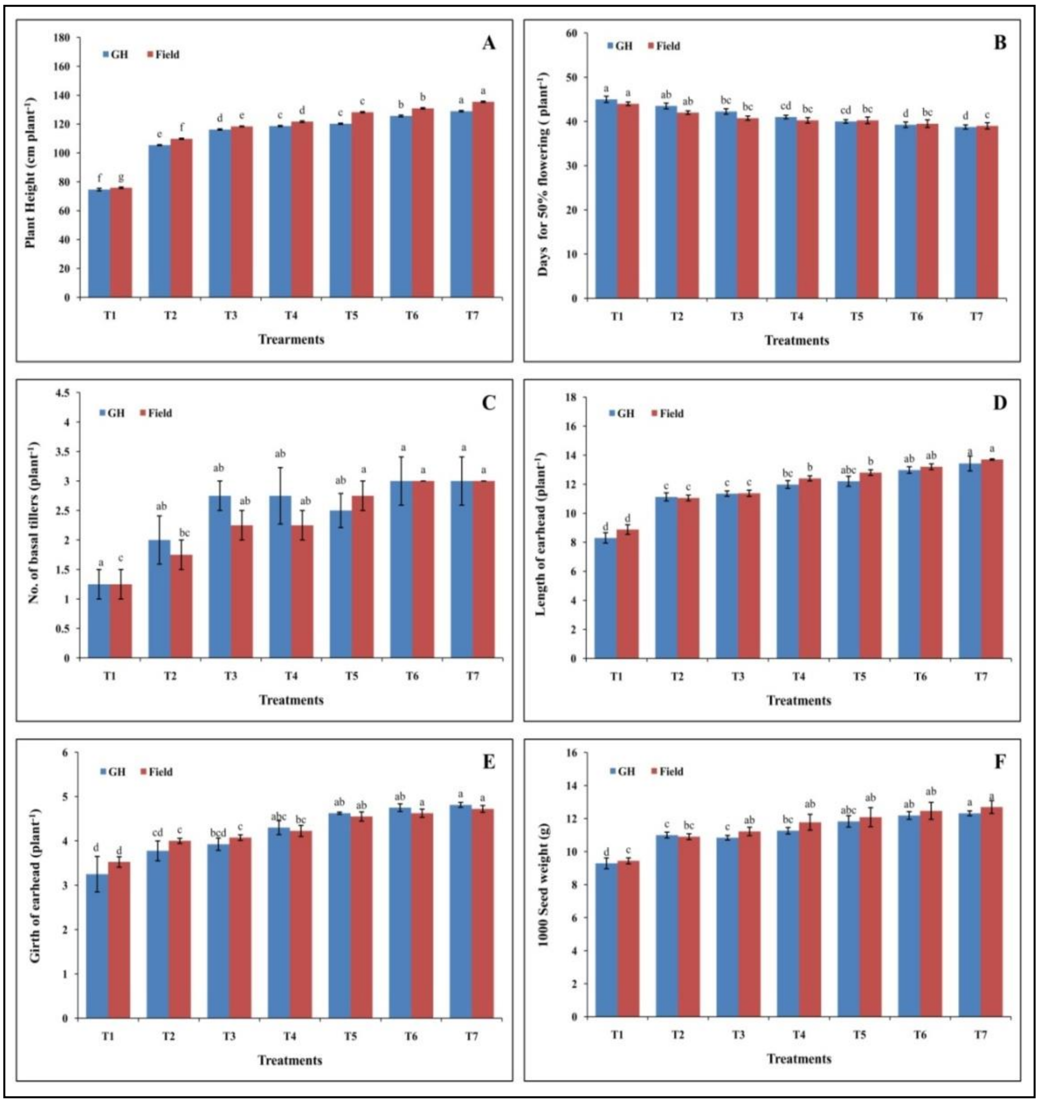

Figure 1: Effect of different elicitor treatments on plant growth parameters of pearl millet under greenhouse and field conditions. Each value is the mean for four replicates (n= 4) and bars sharing the same letters are not significantly different ($p \leq 0.05$) according to Tukey's HSD. The vertical bar indicates the standard error.

Evaluation of IDM module for blast disease protection in pearl millet under greenhouse and field conditions

The disease protection studies upon different treatment showed a significant difference in disease protection compared to control plants (Table 2). The seeds treated with the *P. fluorescens* showed a disease protection of 62.75% and 59.25%, while BABA offered 61.75% and 60% under greenhouse and field conditions, respectively. The combined treatment of *P. fluorescens* and BABA showed significant enhancement in disease protection (69.75% and 67.25% under greenhouse and field conditions, respectively) compared to individual treatments. The chemical fungicide (tricyclazole) offered disease protection of 91.25% and 86.5%, while the formulation of tricyclazole and BABA offered 91.75% and 88% disease protection under greenhouse and field conditions, respectively. From the results, it is affirmed that the treatment of selected elicitors (*P. fluorescens* and BABA) was effective in inducing resistance to *M. grisea* the causal agent of pearl millet blast disease effectively under both greenhouse and field conditions (Fig. 2).

Table 2: Effect of different treatments on disease protection in pearl millet against blast disease under greenhouse and field conditions

Treatment No.	Disease Protection (%)	
	Greenhouse	Field
T1	0.75 ± 0.25^{d}	0.50 ± 0.28^{e}
T2	62.75 ± 0.25^{c}	59.25 ± 0.47^{d}
T3	61.75 ± 0.47^{c}	60.00 ± 0.91^{d}
T4	69.75 ± 0.75^{b}	67.25 ± 0.62^{c}
T5	91.25 ± 0.62^{a}	86.50 ± 0.64^{b}
T6	91.75 ± 1.03^{a}	88.00 ± 0.40^{ab}
T7	92.50 ± 0.86^{a}	89.75 ± 0.47^{a}

Each value is the mean for four replicates (n= 4) and ± indicates standard errors. Means followed by the same letter(s) within the same column are not significantly (*p ≤ 0.05*) different according to Tukey's HSD.

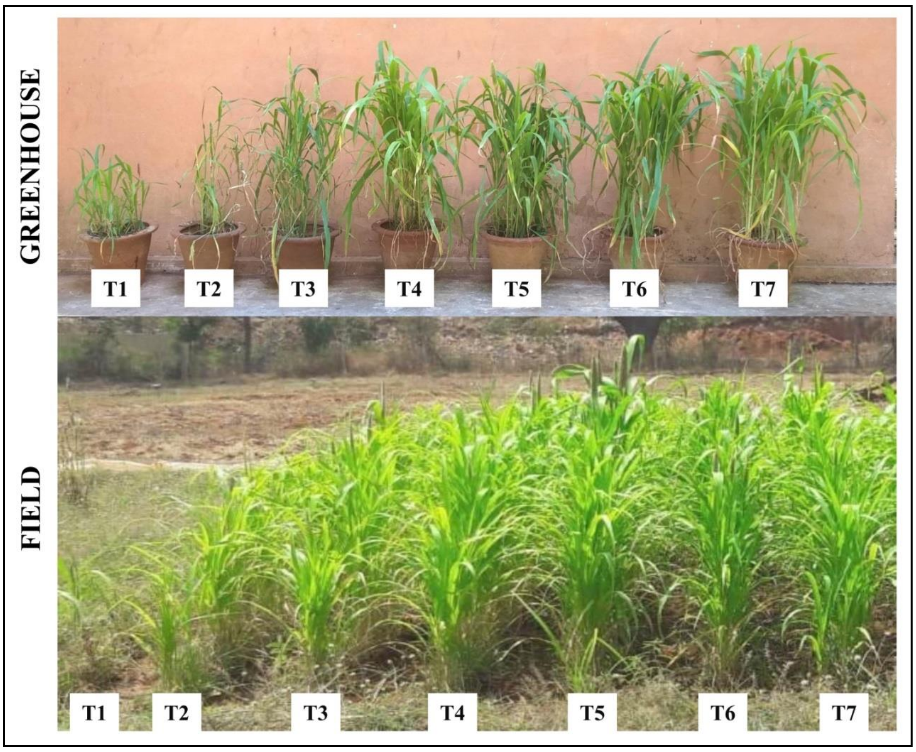

Figure 2: Effect of seed treatment with selected elicitors on the protection of blast disease in pearl millet under greenhouse and field conditions.

DISCUSSION

Eco-friendly and sustainable management of plant diseases is one of the major priorities of present-day agriculture. Aiming higher yield and to control of plant pathogens, several synthetic pesticides and fertilizers are being used in large-scale, but this has eventually caused environmental hazards that led to many concerns [14]. As a result, there is a continuous search for newer, novel and ecologically safe methods for disease management. It has been proved that several less toxic chemicals and some biotic agents are involved in tackling the diseases of many crop plants [15-16]. The efforts in optimizing the benefits of bio-agents, chemicals and other cultural practices with their suitable combined proportions have given rise to the idea of integrated disease management (IDM) strategies [17]. IDM involves the usage of all disease management agents or/and practices in an ecologically balanced and economically affordable manner and the whole idea is based on to strive towards sustainable agriculture. The current study involved seed treatment with selected elicitors (*P. fluorescens* and BABA) individually and in combinations along with appropriate control. A total of seven different treatments (T1 to T7) were evaluated and all the treatments significantly enhanced the plant growth parameters compared to control. From the results it was observed that among the two different elicitors evaluated, BABA was most effective in enhancing the plant growth parameters compared to *P. fluorescens*, while the combination of both the elicitors was significantly effective in improving the growth parameters compared to their specific treatments. The results are by the findings of other researchers wherein, single treatment (biotic or abiotic) was less effective in enhancing the plant growth parameters compared to a combination of the same in rice and tomato and shallot [4,6,18]. The present results confirm that the combination of the treatments is more effective in enhancing plant growth parameters which are in agreement with the findings of Daroga et al. [19] and Anith et al. [20].

Further, the treatments were evaluated for their efficacy to induce disease resistance in pearl millet to blast disease caused by *M. grisea*. The results of the study indicate that among the seven different treatments evaluated, T7 (resistant) offered maximum disease protection of 92.5% followed by T6 (BABA + Tricyclazole) and T5 (Tricyclazole), which contributed 91.75% and 91.25% protection, respectively. Our results are in corroboration with the studies of Shyamala and Sivakumaar [21], wherein combined treatment (*P. fluorescens* and salicylic acid) effectively controlled the rice blast disease compared to single treatment under both greenhouse and field conditions. Likewise, Jaiganesh [8] reported that bioformulation with *Pseudomonas fluorescens* (PFS), nicotinic acid and panchakavya effectively induced disease resistance in rice crop against blast disease and also enhanced the plant growth parameters both under the greenhouse and field conditions. Also, Varma et al. [22] reported that the treatments involving tricyclazole had given excellent results compared to the farmer's practice in the management of rice blast disease which is in line with the results obtained from our studies. The results firmly indicate that the elicitors evaluated in the study have the potential to induce disease resistance in pearl millet against blast disease both under greenhouse and field conditions.

CONCLUSIONS

The present study highlights the effect of elicitors (both biotic and abiotic) in induction of resistance and enhanced plant growth in pearl millet against the invasion of M. grisea the causal agent of blast disease. It was noted that the individual treatment of *P. fluorescens* and BABA were significantly effective in inducing resistance, but the combination of both the elicitors were more effective than the individual treatments. From the results it can be interpreted that the selected elicitors were effective against blast disease in pearl millet and can be used for sustainable agriculture.

ACKNOWLEDGMENTS

The first author greatly acknowledges University Grants Commission (UGC), New Delhi, for providing FDP-Teacher Fellowship under XII plan. The authors also acknowledge the support provided by ICAR-AICPMIP, Mysore Centre and Department of Studies in Botany, University of Mysore, Mysuru for providing necessary infrastructure to carry out this research work.

REFERENCES

[1] Bale J.S., Van Lenteren J.C., and Bigler, F., Biological control and sustainable food production. Philos. Trans. Royal Soc. B, 363 (1492): 761-776, (2007)

[2] Khoury W.E., and Makkouk, K., Integrated plant disease management in developing countries. J. Pl. Pathol., 92 (4 Supplement): S4 35-S4 42, (2010).

[3] Knight A.L., Codling moth areawide integrated pest management. In: Koul G. Cuperus., N. Elliot. (Eds.) Areawide Pest Management: Theory and Implementation. CAB International, Oxfordshire, UK 2008, pp. 159-190.

[4] Ramasamy S., and Ravishankar M., Integrated pest management strategies for Tomato under protected structures. In: Wakil W., Brust G.E., Perring T.M. (Eds.) Sustainable Management of Arthropod Pests of Tomato. Academic Press, US 2018, pp. 313-322.

[5] Rotolo C., Angelini R.M.D.M., Dongiovanni C., Pollastro S., Fumarola G., Di Carolo M., Perrelli, D., Natale P., and Faretra, F., Use of biocontrol agents and botanicals in integrated management of *Botrytis cinerea* in table grape vineyards. Pest Manag. Sci., 74 (3): 715-725, (2018)

[6] Surulirajan M., and Kandhari J., Screening of *Trichoderma viride* and fungicides against *Rhizoctonia solani*. Ann. Pl. Prot. Sci., 11 (2): 382-384, (2003).

[7] Khan A.A., and Sinha A.P., Influence of formulations doses and time of application of fungal bio-agents on rice sheath blight. Ann. Pl. Prot. Sci., 14 (1): 157-161. (2006).

[8] Jaiganesh V. Integrated management of blast diseases in rice. J. Biopest., 11 (2): 82-88, (2018)

[9] Jukanti A.K., Gowda C.L.L., Rai K.N., Manga V.K., Bhatt R.K., Crops that feed the world 11. Pearl millet (*Pennisetum glaucum* L.): an important source of food security, nutrition and health in the arid and semi-arid tropics. Food Sec., 8: 307-329, (2016).

[10] Wilson J.P., and Gates, R.N., Forage yield losses in hybrid pearl millet due to leaf blight caused primarily by *Pyricularia grisea*. Phytopathol., 83 (7): 739-744, (1993)

[11] Baysal O., Laux P., Zeller W., Further studies on the induced resistance (IR) effect of plant extract from *Hedera helix* against fire blight (*Erwinia amylovora*). In: Hale C., Mitchell R. (Eds.) Proc. 9th Int. Workshop on Fire Blight. Acta Hort., 590: 273-277, (2002)
[12] Joshi H.D., and Gohel N.M., Management of blast [*Pyricularia grisea* (Cooke) Sacc.] disease of pearl millet through fungicides. The Bioscan., 10, 1855-1858, (2015)
[13] Gaikwad A.P., and Balgude Y.S., Induction of systemic resistance in rice against blast disease by bioagents and chemicals. J. Rice Res., 2 (9): 63-66, (2016)
[14] Burketova L., Trda L., Ott P.G., and Valentova O., Bio-based resistance inducers for sustainable plant protection against pathogens. Biotechnol. Adv. 33 (6): 994-1004, (2015)
[15] Aldesuquy H., and Ghanem H., Exogenous salicylic acid and trehalose ameliorate short term drought stress in wheat cultivars by up-regulating membrane characteristics and antioxidant defense system. J. Hortic., 2: 139, (2015).
[16] Amer M.A., El-Abd S.M., Deraz S.F., and Zaid, N.A. Systemic acquired resistance induced by some biotic agents against downy mildew of cucumber disease. International Conference on Plant, Marine and Environmental Sciences (PMES-2015) Jan. 1-2, 2015 Kuala Lumpur (Malaysia) (2015).
[17] Varaprasada Rao Ch., and Anil Kumar P., Evaluation of fungicides, biocontrol agents and plant extracts against rice blast caused by *Pyricularia grisea* (Sacc.) under field conditions. Int. J. Curr. Microbiol. App. Sci., 7 (04): 3855-3861, (2018)
[18] Dinakaran D., Gajendran G., Mohankumar S., Karthikeyan G., Thiruvudainambi S., Jonathan E.I., and Miller S., Evaluation of integrated pest and disease management module for shallots in Tamil Nadu, India: A farmer's participatory approach. J. Integr. Pest Manag., 4 (2): B1-B9, (2013).
[19] Daroga Singh, Singh V.K., Gopal Singh, and Singh U.S., Integrated management of sheath blight and sheath rot by using *Trichoderma viride* and Validamycin in rice cv. Pusa Basmati-1. Progressive Agric. 8 (1): 181-182, (2007).
[20] Anith K.N., Momol M.T., Kloepper J.W., Marois J.J., Olson S.M., and Jones J.B., Efficacy of plant growth-promoting rhizobacteria, acibenzolar-S-methyl, and soil amendment for integrated management of bacterial wilt on tomato. Pl. Dis., 88 (6): 669-673, (2004)
[21] Shyamala L., and Sivakumaar P.K., Integrated control of blast disease of rice using the antagonistic rhizobacteria *Pseudomonas fluorescens* and the resistance inducing chemical salicylic acid. Int. J. Res. Pure Appl. Microbiol. 2 (4): 59-63, (2012)
[22] Varma C.Y., Santhakumari, P., and Mary, C.A., Improving efficacy of fungicides with integration of plant products and resistance inducers for the management of rice blast. J. Mycol. Pl. Pathol., 43(1), 37-43, (2013)