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Increased efficacy of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) against leaf folder, *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Pyralidae), incidence on silica enriched rice crop

I. Paramasiva[1]*, T. Murali Krishna[2], Ch. Sreelaksmi[3], U. Vineetha[4] and P. Madhusudan[5]

[1]Department of Entomology, Agricultural Research Station, Akuthota, Muthukur Road, Nellore 524 003, A.P., India
[2]Krishi Vigyan Kendra, Rastakuntubai 535 523, Vizianagaram Dist., A.P., India
[3]Department of Genetics and Plant breeding, Agricultural Research Station, Nellore, A.P., India
[4]Department of Agronomy, Agricultural Research Station, Nellore, Andhra Pradesh, India
[5]Department of Plant Pathology, Agricultural Research Station, Nellore, A.P., India

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ABSTRACT

A possible synergistic effect between biological control agent *Beauveria bassiana* (Bb) and potassium silicate (K_2Sio) for control of leaf folder on rice was investigated at Agricultural Research Station, Nellore (ANGRAU, Guntur, Andhra Pradesh) under field conditions. Three sequential Silicon application in the form of potassium silicate (KSil) was given as foliar spray @ 80 mg/L, when leaf folder population density reached to ETL, *B. bassiana* @ 1.3x106 conidia/ml application was carried out. Two sequential applications of potassium silicate (two weeks after transplantation and at active tillering stage) + B. bassiana caused leaf folder population reduction of 42.80 per cent over control at 8 days after spraying. This reduction was significantly higher than the mortalities caused by the two single treatments alone (*B. bassiana*: 17.55 %; Ksil @ 2sprayes: 8.83). This study providing strong evidence of a synergistic effect of the combination treatment on leaf folder mortality. Similarly, three sequential applications of potassium silicate (two weeks after transplantation, at active tillering stage and PI stage) + *B. bassiana* caused 41.88 % reduction in leaf older population over control at 8 days after spraying, whereas three sequential applications of potassium silicate and application of *B. bassiana* alone caused 20.57 and 17.55 % reduction in leaf folder incidence, respectively. Indicates application of potassium silicate and *B. bassiana* alone did not gave as satisfactory results as used in combinations. In the present study the sequential applications of Potassium silicate + *B. bassiana* gave more promising control of rice leaf folder compared to the application of *B. bassiana* alone, proving the synergistic effect of the potassium silicate + *B. bassiana* combination on leaf folder incidence.

Key words: *Potassium silicate, Beauveria bassiana, Synergism, Rice leaf folder, Cnaphalocrocis medinalis.*

([1]Scientist (Ento.), [2]Programme Coordinator)

Introduction

India is the world's second-largest producer of rice, and the largest exporter of rice in the world. Production increased from 53.6 million tons in FY 1980 to 120 million tons in FY 2020-21. Rice (*Oryza sativa* L.) ranks 1st in terms of area harvested and has important role in the economy of India. There are number of factors which lead to the reduction in rice yield, among these losses caused by insect pests varies from 20-30% annually (Chatterjee *et al.*, 2016, Chaudari *et al*., 2017).

Cnaphalocrocis medinalis Guenee (Lepidoptera: Pyralidae), rice leaf folder was considered as pests of minor importance have increased in abundance in late 1980's and have become major pests in many parts of India. The yield loss is from 30 to 80 per cent due to leaf folder epidemic situation (Nanda and Bisoi, 1990; Shah *et al.*, 2008). An increase in leaf folder population could be attributed to the large scale cultivation of high yielding varieties, application of fertilizers, and continuous use of insecticides leading to outbreak of this pest in several countries, including India (Khan *et al*., 1988; Shanmugam *et al*., 2006; Kaushik, 2010). The larvae fold the leaves longitudinally by stitching the leaf margins and feed by scraping the green mesophyll tissue from within the folded leaves. This feeding causes linear, pale white stripes that result in white patches. The heavy use of insecticides and high nitrogen fertilizer rates seem to favors leaf-folder population outbreaks (Subbaih and Morachan, 1974; Mahadev *et al*., 1995; Sinclair, 1998 and Paramasiva *et al.*, 2020).

Over reliance and indiscriminate use of highly toxic and hazardous pesticides has resulted in pest resistance, resurgence and environmental pollution leading to imbalance in natural ecosystem. Hence the use of alternate eco-freindly strategies like microbial insecticides and use of micronutrients to induce host plant resistance is a more appropriate approach to suppress pest population.

Beauveria bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a significant natural pathogen of insect and can infect more than 700 species of arthropods (Moraes *et al.*, 2005). These fungi infect their hosts by penetrating through the cuticle, gaining access to the haemolymph, producing toxins, and grow by uilizing nutrients present in the haemocoel to avoid insect immune responses (Hajeck and Leger, 1994). Entomopathogenic fungi may be applied in the form of conidia or mycelium which sporulates after application (Hoy, 1999). Their hosts comprise of numerous pests and its large distinction in virulence towards different insect hosts makes it one of the more resourceful entomophagous fungi for the biological control of insect pests (Rehner, 2005)

Silica content in the plant is reported to play an important role in strengthening the cell walls of plants (Painter, 1951) and enhances resistance to both pests and diseases in the field (Qin and Tian, 2004; Ma, 2004). It has been reported that silicon suppresses insect pests in rice such as stem borer, leaf folders and leaf hoppers (Takahashi, 2002). The filed application of 10 t/ha calcium silicate resulted in a reduction of 30 % borer damage reported in sugarcane. The most susceptible varieties of sugarcane showed the highest silicon uptake and the greatest response (Keeping and Meyer, 2003), application of potassium silicate (KSil) as a source of Si, has been reported to suppress insect pests such as borers, sap suckers and mites (Keeping and Kvedaras, 2008; Liang *et al*., 2006).

The soluble silicon absorbed by plants acts as a catalyst in the plants pest resistance mechanisms. Hence, pests feeding on silicon positive plants will struggle to feed without interference by natural plant resistance compounds. As such these pests are likely to be stressed and therefore will be more susceptible to bio-control agents than pests feeding on silicon negative plants. Therefore we considered whether Potassium silicate (KSil) as a source of Si could synergize the fungal bio-control agent Beauveria bassiana (Bb) for control of rice leaf folder. In most of the Poaceae (Gramineae) plants silicon occurs in the epidermis, which might dislodge young larvae before they can establish. Silicon increases the hardness of plant tissue, which interferes with insect larval boring and feeding activity.

Therefore the aim of this study was to investigate whether Si enriched rice crop by means of sequential applications of Potassium silicate would increase the control efficacy of *B. bassiana* against leaf folder infestations.

Materials and Methods

A field experiment was conducted to investigate the combined effect of potassium silicate and *B. bassiana* against rice leaf folder, *C. medinalis* under field conditions.

Field preparation and experimentation

Field was prepared according to the paddy field requirements. Irrigation and fertilizer was applied constantly to meet the crop nutrient requirements adequately. There were nine treatments, T_1 : *Beauveria bassiana* @ 1.3x10^6 conidia/ml; T_2 : Si @ 80 mg/l at 2 wks after transplantation; T_3 : T_2 + *Beauveria bassiana* @ 1.3x10^6 conidia/ml; T_4: Si @ 80 mg/l at 2 wks after transplantation and at active tillering stage; T_5 : T_4+ *Beauveria bassiana* @ 1.3x10^6 conidia/ml; T_6: Si @ 80 mg/l at 2 wks after transplantation and at active tillering stage and at Panicle initiation stage T_7 : T_6 + *Beauveria bassiana* @ 1.3x10^6 conidia/ml; T_8: Chlorpyriphos; T_9 : Untreated control (Water spray). All the agronomic practices were followed uniformly in all the plots. The experiment was carried out in Randomized block design (RBD) with three replications.

Silicon application in the form of potassium silicate (K_2Sio_3) was given as foliar spray @80 mg/L at two weeks after transplantation for T2, T4 and T6 plots; at two weeks after transplantation and at active tillering stage for T4 and T6 plots and at two weeks after transplantation, at active tillering stage & at panicle initiation stage (PI) for T6 plot. The incidence of *C. medinalis* was recorded at regular time intervals in each experimental plot and pre treatment data was recorded. When leaf folder population density reached to ETL, *B. bassiana* @ 1.3x106 conidia/ml application was carried out with the help of hand sprayer in order to suppress the leaf folder population. The post treatment data on leaf folder damage was recorded at three and eight days after imposition of treatments.

Assessment of rice leaf folder per cent incidence and yield

Observations on leaf damage by the leaf folder was assessed by counting the total number of leaves and number of leaf folder damaged leaves on 20 random hills per plot at one day before and 3 and 8 days after imposition of treatments by leaving the border lines form sides. The per cent leaf folder incidence damage was computed as follows.

$$\text{Per cent leaf folder damage} = \frac{\text{Number of damaged leaves/hill}}{\text{Total number of leaves/hill}} \times 100$$

The crop was harvested when 90% of the grain matured in all the treatments. The grain yield of each plot were recorded separately by leaving the border rows and computed to quintals per hectare.

Potassium silicate and fungal application

B. bassiana conidial liquid containing 1.3x10^6 conidia ml^{-1} were formulated and provided by The ICAR-National Bureau of Agricultural Insect Resources, Bengaluru. Potassium silicate (K_2Sio_3) (Sio_2 : K_2O 2.5:1 wt%) was provided by Alfa Aesar, United states used as source of soluble silicon (Si).

Statistical analysis

The data was analyzed statistically by using SPSS 13.0 package. The means were compared by Duncan's multiple range test (DMRT) for their significance at 5% probable level.

Results and Discussion

All the tested treatments were superior over the untreated control at 3 and 8 days after imposition of treatments. At three days after imposition of treatments the results were non-significant indicates there is no significant difference among the treatments including untreated check. At eight days after imposition of treatments lowest per cent leaf damage caused by *C. medinalis* was observed with the T_7 treatment, i.e three foliar applications of potassium silicate (at 2 weeks after transplantation, at active tillering stage and at PI stage) + *B. bassiana* application (15.90 %) and was followed by T_5 treatment, i.e two foliar applications of potassium silicate (at 2 weeks after transplantation and at active tillering stage)+ *B. bassiana* (18.04 %) and three foliar applications of potassium silicate (20.67%). Leaf folder incidence was highest (33.62 %) in the control treatment followed by two sequential applications of potassium silicate (29.13 %).

Interestingly with three foliar applications of potassium silicate 20.67 % leaf folder incidence was recorded where as with chlorpyriphos application 22.50 % leaf folder damage was noticed. With the application of *B. bassiana* alone 25.13 % leaf folder damage was recorded and with single application of potassium silicate 24.92 % leaf folder damage was recorded compared to untreated check (33.62 %).

Percent reduction

Two sequential applications of potassium silicate (two weeks after transplantation and at active tillering stage) + *B. bassiana* caused leaf folder population reduction of 42.80 per cent over control at 8 days after spraying. This reduction was significantly higher than the mortalities caused by the tow single treatments alone (*B. bassiana*: 17.55 %; Ksil @ 2 sprayes: 8.83). This study providing strong evidence of a synergistic effect of the combination treatment on leaf folder mortality. Similarly, three sequential applications of potassium silicate (two weeks after transplantation, at active tillering stage and PI stage) + *B. bassiana* caused 41.88 % reduction in leaf older population over control at 8 days after spraying, where as three sequential applications of potassium silicate and application of *B. bassiana* alone caused 20.57 and 17.55 % reduction in leaf folder incidence, respectively. Indicates application of potassium silicate and *B. bassiana* alone did not gave as satisfactory results as used in combinations (Gatarayiha *et al*., 2010 and Jiang *et al*., 2012). Similar synergistic effects were observed against Colorado potato beetle by applying entomopathogenic fungi, *B. bassiana* with synergistic chemicals (Anderson *et al.,* 1989).

KSil application as a source of Si enhanced the control efficacy of *B. bassiana* in the present study, as measured by highest % reduction in leaf folder incidence when *B. bassiana* was applied after sequential applications of KSil. The role of Si in enhancing *B. bassiana* efficacy in this study was plant-mediated rather than by direct effect on leaf folder (Gatarayiha *et al.,* 2010). The aforementioned observations support our hypothesis that Si applied in the form of potassium silicate increases the resistance of plants against leaf folder which may reduce the feeding of leaf folder and make it more vulnerable to *B. bassiana* infection.

Hassan and Charnely, 1989 also studied the combined applications of entomopathogens and sublethal dosages of synthetic insecticides, where synergistic and additive effects leading to increased mortality were observed by the combined application of imidacloprid and *Metarhizium anisopliae*. Shakir *et al*., 2015 also reported maximum mortality (61.91 %)of *Cnaphalocrocis medinalis* was achieved with combined application of potassium silicate, *B. bassiana* and imidacloprid.

Application of *B. bassiana* alone and three sequential applications of potassium silicate resulted in a comparable reduction in leaf folder population 17.55 and 20.57 per cent, respectively. Indicates application of Si alone also had a significant effect on leaf folder control. Djamin and Pathak (1967) found that the growing of rice in silica impeded feeding and boring by the striped rice borer, *Chilo suppressalis* Walker, and reduced larval survival and the number of dead hearts, similarly, Goussain *et al.*, 2002 found that fall armyworm larvae, *Spodoptera frugiperda* displayed inhibited feeding on maize plants fertilized with sodium silicate, suffering increased mandibular wear, higher mortality and cannibalism. Silicon application in paddy seedlings improved grwth and suppressed pest population (Yoshihar *et al*., 1979; Epstein, 1999 and Ma, 2004). Application of potassium silicate and other silicon compounds suppress

Table 1. Efficacy of *Beauveria bassiana* in combination with Potassium silicate against rice leaf folder (pooled data of 2017 and 2018)

Treatments		% leaf folder incidence			% reduction over control	Yield (Q/ha)
		1 DBS	3 DAS	8 DAS		
T1	*Beauveria bassiana* @ 1.3x10^6 conidia/ml	27.77	26.80	25.13	17.55	36.33
T2	Si @ 80 mg/l at 2 wks after transplantation	25.38	31.11	24.92	10.54	35.77
T3	T_2 + *Beauveria bassiana* @ 1.3x10^6 conidia/ml	28.67	21.54	27.29	13.29	34.07
T4	Si @ 80 mg/l at 2 wks after transplantation and at active tillering stage	29.11	24.26	29.13	8.83	37.51
T5	T_4+ *Beauveria bassiana* @ 1.3x10^6 conidia/ml	28.73	21.96	18.04	42.80	47.00
T6	Si @ 80 mg/l at 2 wks after transplantation and at active tillering stage and at Panicle initiation stage	23.71	19.00	20.67	20.57	34.60
T7	T_6 + *Beauveria bassiana* @ 1.3x10^6 conidia/ml	24.92	22.88	15.90	41.88	47.82
T8	Chlorpyriphos	27.60	29.09	22.50	25.74	48.90
T9	Untreated control (Water spray)	30.63	27.88	33.62	0	33.30
	Sig	NS	NS	**		**
	P-value	0.983	0.851	0.001		0.000

the population densities of insect pests such as sap suckers, borers and folivores and non insect pests like mites (Savant *et al.*, 1997; Laing *et al.*, 2006; Keeping and Kvedaras, 2008).

Yield

Untreated control plots suffered a higher leaf folder infestation and lead to a reduction in the economic yield. All other treatments especially three sequential applications of potassium silicate + *B. bassiana* and two sequential applications of potassium silicate + *B. bassiana* proved their efficacy in exerting stress over rice leaf folder incidence and minimizing losses to enhance yield with 48.9, 47.82 and 47.0 Q/ha, respectively. Lowest grain yield of 33.3 quintals/ha was recorded in untreated check and it is followed by with T_2 treatment i.e single application of potassium silicate at 2 weeks after transplantation + *B. bassiana* application (34.07 Q/ha).

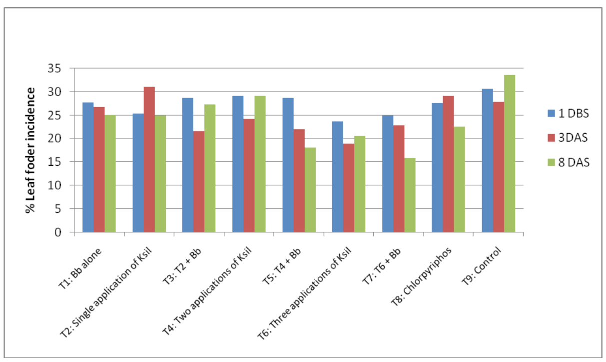

Fig. 1. Efficacy of *B. bassiana* in combination with Potassium silicate against rice leaf folder

The combined effect of two/three sequential applications of potassium silicate + *B. bassiana* as good as the chemical treatment not only in reducing the leaf folder incidence but also in producing higher yields. Rodrigues *et al.*, 2003 revealed that rice depends on the availability of silicic acid at all phases of its growth as well as protection from biotic stresses. Similarly our results showed that potassium silicate is an essential element for the production of rice crop which not only provide a nutrients to the crop but it also play an essential role in the activation of defence mechanism.

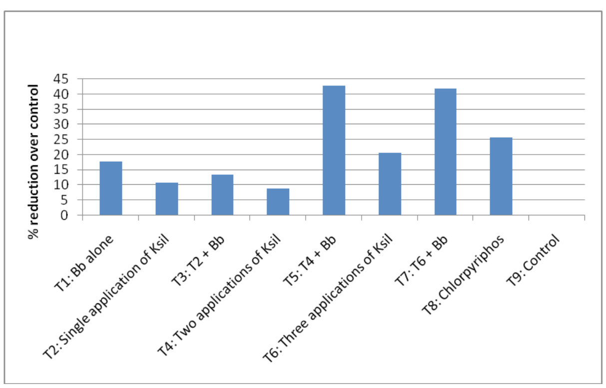

Fig. 2. Percent reduction in leaf folder incidence with Potassium silicate and *Beauveria bassiana* in combination

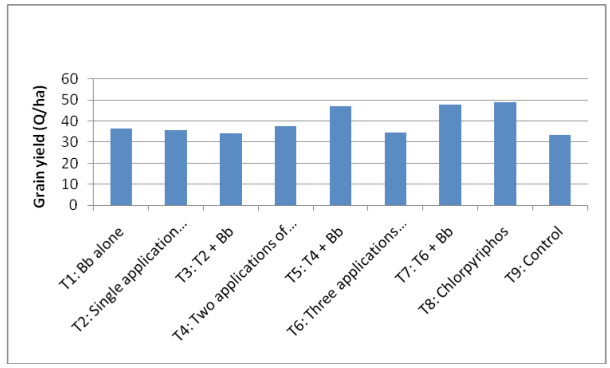

Fig. 3. Combined effect of Potassium silicate and *Beauveria bassiana* on grain yield (Q/ha)

Current study revealed that integration of *B. bassiana* along with potassium silicate increase the efficacy after different exposure time intervals and significantly reduced the infestation losses as well as increased yield which showed the compatibility of entomopathogenic fungi with potassium silicate. Because potassium silicate and *B. bassiana* alone did not gave as satisfactory results as used in combinations.

However, it has to be emphasized that although the combination of *B. bassiana* and KSil significantly reduced the leaf folder incidence, the control was not sufficiently adequate and is needed to be improved with much research.

Conclusion

There is an urgent need for environmentally safe alternatives for the synthetic insecticides which are in use today for the control of rice leaf folder. *B.bassiana* is a significant entomopathogenic fungi that can be used as biological control of insect pests. Even though *B.bassiana* have already been successfully adopted in large-scale and their use is increasing yearly they suffer major limitations. In fact, different abiotic factors, such as UV radiation, low humidity and high temperature, hamper fungal biological activity and, in turn, their effectiveness as biocontrol agents. To overcome this limitation, a possible strategy to improve fungal bio-efficacy, use of micronutrients like si to induce host plant resistance, is a more appropriate approach to suppress pest population. In present study the sequential applications of Potassium silicate + *B. bassiana* gave more promising control of rice leaf folder compared to the application of *B. bassiana* alone, proving the synergistic effect of the potassium silicate + *B. bassiana* combination on leaf folder incidence, the present data is very useful to the development of an eco-friendly control measures.

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