*Indian Journal of Agricultural Sciences* **87** (8): 1099–1103, August 2017/Article
https://doi.org/10.56093/ijas.v87i8.73280

# Field evaluation of endophytic entomopathogenic fungi against maize stem borer (*Chilo partellus*) (Crambidae: Lepidoptera)

B RAMANUJAM[1], KRISHNA JAPUR[2], B POORNESHA[3], A N SHYLESHA[4] and R RANGESHWARAN[5]

*ICAR-National Bureau of Agricultural Insect Resources, H A Farm post, Bellary Road, Hebbal, Bengaluru Karnataka, 560024.*

Received: 17 January 2017; Accepted: 27 February 2017

ABSTRACT

The present study was carried out to assess the field efficacy of endophytic isolates of *Beauveria bassiana* (Bb-5a, Bb-23 and Bb-45) and *Metarhizium anisopliae* (Ma-35) against maize stem borer (*Chilo partellus* Swinoe) during *kharif* seasons of 2015 and 2016 at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Attur Farm, Bengaluru, Karnataka. All the tested isolates showed suppression of maize stem borer damage during both seasons. Pooled data indicated that lesser dead hearts (9.5-12.71%), stem tunnelling (3.98-4.36 cm/plant) and exit holes (2.59-2.79 /plant) in the treated plants compared to untreated control which showed 21.38% dead hearts, 8.01 cm/ plant of stem tunnelling and 6.99 exit holes/plant. Among the four isolates tested, Bb-5a isolate of *B. bassiana* and Ma-35 isolate of *M. anisopliae* showed significantly superior effect in lowering the incidence of dead hearts (9.50 and 12.71%), stem tunnelling (3.98 and 4.36 cm/plant) and exit holes (2.59 and 2.79/plant) with higher yields of 72 and 70.0 tonnes/ha respectively.

**Key words:** *Beauveria bassiana*, Biological control, *Chilo partellus*, Endophyte, Entomopathogenic fungi, Maize stem borer, *Metarhizium anisopliae*

Maize is the one of the important cereal crops of India as a human food as well as animal feed, occupying nearly 37 per cent of area under cultivation with the annual production of 225 lakh tonnes (Anon. 2016) . Maize stem borer (*Chilo partellus* Swinhoe) (Crambidae: Lepidoptera) is one of the most important insect pests of maize (*Zea mays* L.) in India. The crop losses caused by this pest ranges from 24 to 83 percent (Sarup *et al*. 1987). *Chilo partellus* infests the crop throughout its growth from seedling stage to maturity and causing 90-95% loss in grain yield (Jalali and Singh 2002). It mainly attacks the crop during *kharif* season and has been reported from Asom, Andhra Pradesh, Bihar, Gujarat, Haryana, Himachal Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Uttar Pradesh. Depending on the ecological zone, the severity of the damage varies. In India, the pest also infests other crops like, sorghum, finger millet, sugarcane, pearl millet, rice and wheat and cause considerable damage (Jalali and Singh 2003). *C. partellus* is also a major pest of maize in various parts of Africa and southern Asia.

The attack of this insect on maize plants begins with the laying of eggs on the leaves of inner whorl, which hatch and the neonate larvae move into the leaf whorls where they feed and develop on the leaf bases, causing lesions. When the larvae develop to the late third or early fourth instar, they bore into the stem feeding on the tissues and making tunnels. When the larvae feeds within the leaf whorl or stem, cut through the growing tissues leading to drying up of central leaves to produce the "dead heart" symptoms resulting in death of the plant.

Control of stem borers by chemical insecticides is extremely difficult because of the cryptic life cycle of the pest. The shift from conventional synthetic insecticides to biological control agents has been due to environmental concerns and difficulties with insecticides resistance, thus entomopathogenic fungi (EPF) are virtually considered to be safe and eco-friendly insect control agents (Miranpuri and Kachatourian 1993).

Earlier studies revealed that, promising strains of *B. bassiana* (NBAII-Bb-5a, 23 and 45) and *M. anisopliae* (NBAII-Ma-35) were identified against *C. partellus* in the laboratory bioassay (Renuka *et al.* 2015, Ramanujam *et al.* 2015). These promising strains of *B. bassiana* were established as endophytes in maize stem and leaf tissues

[1]Principal Scientist (e mail: bonamramanujam58@gmail.com), Division of Insect Ecology, [2,3]Senior Research Fellow (e mail: krishna3944@gmail.com, poorniinbiotech@gmail.com), [4]Principal Scientist (e mail: shyleshaan.nbaii@gmail.com,) Division of Insect Ecology, [5]Principal Scientist (e mail: rangeshw@rediffmail.com, Division of Molecular Entomology

by artificial inoculation through foliar sprays of conidial suspensions (Renuka *et al.* 2016, Ramanujam *et al.* 2016). The present studies were undertaken to determine the effect of these four endophytic isolates of entomopathogenic fungi on the infestation level of maize stem borer during *kharif* season.

## MATERIALS AND METHODS

The field trial against stem borer, *C. partellus* was carried out at ICAR-NBAIR Attur Farm, Bengaluru, Karnataka, India, during *kharif* seasons of 2015 and four promising isolates of endophytic entomopathogenic fungi (*Beauveria bassiana* Bb-5a, Bb-23, Bb-45 and *Metarhizium anisopliae* Ma-35).

The field trials were laid out in completely randomised block design with 5 treatments and replicated four times with popular maize hybrid, Nityashree (NAH-2049) which is susceptible to stem borer attack was selected for the study. The maize seeds were dibbled manually in the experimental plot with a plot size of 5m×6 m for each replication and spacing of 60 cm × 30 cm were maintained. All the agronomic practices with recommended doses of fertilizers were followed to maintain good plant health till harvest of crop as per package of practice by UAS, Bengaluru, Karnataka and harvesting was done manually.

The oil formulation of all the isolates were diluted in water (5 ml/l) and applied twice with the help of hand sprayer. Treatments application of endophytic entomopathogenic fungal isolates at the spore dose of $1 \times 10^8$ cfu/ml were given at 15 and 30 days after germination in the experimental field during both years.

A week after second spray, laboratory-reared *C. partellus* larvae of 2$^{nd}$ instar ten/plant were released carefully into the inner leaf whorl with a camel hair brush. Observations on the number of dead hearts (DH), extent of stem tunneling (cm/plant) (ST), number of exit holes per plant (EH) and yield data were recorded at harvest by splitting longitudinally from top to the base of the plant. The recorded data were statistically analyzed by using SPSS v16 software and later analyzed data were averaged and separated by Duncan's Multiple Range Test (DMRT). The treatment-wise cob yield/plant was recorded and converted to tonnes/ha.

## RESULTS AND DISCUSSION

Data on percent dead hearts, stem tunnelling/plant (cm), number of exit holes/plant and cob yield during *kharif* season of 2015 were presented in Table 1 and data of 2016 were presented in Table 2. The pooled data analysis of 2015 and 2016 is presented in Table 3.

### *Dead hearts*

The experimental results revealed that during 2015 the dead hearts percentage ranged from 10.22-20.11% in EPF treated plots compared to 23.56% in the untreated control. Among the tested isolates, Bb-5a of *B. bassiana* showed lower dead hearts of 10.22% followed by Ma-35 of *M. anisopliae* (13.33%) which were on par with each other as depicted in Table 1. Similarly during 2016, the dead hearts percentage ranged from 9.0-18.50% in EPF treated plots compared to untreated control which recorded 19.42%. Bb-5a and Ma-35 showed lower dead hearts of 9.0 and 12.08%

Table 1 Effect of different endophytic isolates of EPF on *Chilo partellus* during *kharif* 2015

| Treatment | Isolate | Average No. of dead hearts | Deads heart (%) | No. of exit holes/plant | Stem tunneling/ plant (cm) | Cob yield/ 10 plants (kg) | Cob yield (tonnes/ha) |
|---|---|---|---|---|---|---|---|
| T1 | Bb-5a | 3.07$^{a}$ (1.87) | 10.22 | 1.80$^{a}$ (1.52) | 1.23$^{a}$ (1.32) | 14.20$^{a}$ (3.83) | 78.8 |
| T2 | Bb-23 | 6.03$^{cd}$ (2.55) | 20.11 | 3.60$^{b}$ (2.02) | 3.11$^{b}$ (1.90) | 12.20$^{b}$ (3.56) | 67.7 |
| T3 | Bb-45 | 5.07$^{bc}$ (2.35) | 16.89 | 1.90$^{a}$ (1.55) | 2.53$^{ab}$ (1.74) | 13.60$^{b}$ (3.75) | 75.4 |
| T4 | Ma-35 | 4.00$^{ab}$ (2.12) | 13.33 | 2.10$^{a}$ (1.61) | 1.77$^{ab}$ (1.51) | 13.90$^{a}$ (3.79) | 77.1 |
| T5 | Control | 7.07$^{d}$ (2.74) | 23.56 | 7.20$^{d}$ (2.77) | 5.20$^{c}$ (2.39) | 11.80$^{b}$ (3.51) | 65.4 |
| CD (P = 0.05) | | 1.24 | | 1.21 | 1.85 | 2.05 | |

Means followed by the similar letters in the columns are not significantly different at 5% by DMRT.

Table 2 Effect of different endophytic isolates of EPF on *Chilo partellus* during *kharif* 2016

| Treatment | Isolate | Average No. of dead hearts | Dead heart (%) | No. of exit holes/plant | Stem tunneling/ plant (cm) | Cob yield/ 10 plants (kg) | Cob yield (tonnes/ha) |
|---|---|---|---|---|---|---|---|
| T1 | Bb-5a | 2.70$^{a}$ (1.79) | 9.00 | 3.38$^{a}$ (1.97) | 6.73$^{a}$ (2.69) | 11.8$^{a}$ (3.51) | 65.6 |
| T2 | Bb-23 | 4.25$^{b}$ (2.18) | 14.17 | 5.35$^{ab}$ (2.42) | 9.83$^{b}$ (3.21) | 9.9$^{a}$ (3.22) | 54.6 |
| T3 | Bb-45 | 5.55$^{c}$(2.46) | 18.50 | 5.10$^{ab}$ (2.37) | 8.53$^{ab}$ (3.00) | 10.2$^{a}$ (3.27) | 56.7 |
| T4 | Ma-35 | 3.63$^{ab}$ (2.03) | 12.08 | 3.48$^{a}$ (1.99) | 6.95$^{a}$ (2.73) | 11.4$^{a}$ (3.44) | 62.9 |
| T5 | Control | 5.83$^{c}$ (2.51) | 19.42 | 6.78$^{b}$ (2.70) | 10.83$^{b}$ (3.37) | 10.0$^{a}$ (3.23) | 55.2 |
| CD (P = 0.05) | | 1.10 | | 2.4 | 2.80 | 2.08 | |

Means followed by the similar letters in the columns are not significantly different at 5% by DMRT.

respectively and were on par with each other during 2016 (Table 2). The pooled data of both years revealed that the lowest dead heart percentage were noticed in Bb-5a (9.50%) and Ma-35 (12.71%) which were statistically on par with each other as compared to untreated control plot recorded 21.38% as shown in Table 3. The reduction of dead hearts in the present investigation may be due to endophytic activity of *B. bassiana* and *M. anisopliae* as reported by Renuka *et al*. (2016). Although all the four tested isolates reported to establish as endophytes in maize stem and leaf tissues and translocate in the maize plant (Renuka *et al*. 2016), the isolates of Bb-5a and Ma-35 showed superior suppressive effect against *C. partellus*. Maize plants treated with liquid or granular formulations of *B. bassiana* conidia at the whorl stage of development became internally colonized by the fungus (Lewis *et al*. 1996). Tefera and Pringle (2007) noticed from Ethiopia, Africa in endophytic establishment of *B. bassiana* and *M. anisopliae* in maize with considerable reduction of dead hearts of *C. partellus*, when these isolates were applied as foliar sprays under greenhouse condition. Cherry *et al*. (2004) recorded that seed dressing of maize seeds with endophytic *B. bassiana* strain against *Sesamia calamistis* showed significant reduction of dead hearts by 27% in Republic of Benin in Africa.

*Stem tunnelling*

The experimental results revealed that stem tunnelling ranged from 1.23-3.11 cm/plant during 2015 in the EPF treated plots compared to untreated control which recorded 5.20 cm/plant. The minimum stem tunnelling was noticed in Bb-5a treated plot (1.23 cm/plant) as represented in Table 1. Similar results were also noticed during 2016 as it ranged from 6.73-9.83 cm/plant compared to untreated control which recorded 10.83 cm/plant. The lower stem tunnelling of 6.73 and 6.95 cm/plant were recorded in Bb-5a and Ma-35 respectively which were on par with each other as shown in Table 2. The pooled data represents that the stem tunnelling ranged from 3.98-6.47 cm/plant compared to untreated control which recorded 8.01 cm/plant. The lowest damage were noticed in Bb-5a (3.98 cm/plant) shown in Table 3 (Fig 1). The maximum stem tunnelling of 8.01 cm/plant were recorded in untreated control. The present experiments provide the evidence of decrease in stem tunnelling of *C. partellus* in maize plants by endophytic isolates of EPF. The reduced stem borer activity in maize plants is mainly due to the systematic activity of *B. bassiana* and *M. anisopliae* isolates. The present result supports the observations of earlier workers who reported reduction in stem tunnelling in maize plants due to endophytic EPF. Reddy *et al*. (2009) reported significantly lesser stem tunnelling of *C. partellus* in sorghum plants treated with endophytic *B. bassiana*. Cherry *et al*. (2004) reported efficacy of stem injection of *B. bassiana* conidia ($1\times10^8$) into maize stem which led to reduction of stem tunnel length (7.25 cm) caused by maize stem borer, *Sesamia calamistis* under green house and field conditions. Similar effects were also noticed when maize plants were injected with endophytic *B. bassiana* under field condition resulting in reduction of tunnelling caused by European corn borer, *Ostrinia nubilalis* as reported by Bing and Lewis (1991). The whorl application of granular formulations of endophytic *B. bassiana* against *O. nubalis* in Iowa (USA) was found most effective in reducing the larval tunnelling by 20-53% (Lewis *et al.* 2002).

*No. of exit holes*

The no. of exit holes ranged from 1.80 to 3.60/plant during 2015 in the EPF treated plots compared to untreated control which recorded 7.20/plant. Among the tested isolates the lowest exit holes were recorded in Bb-5a (1.80/ plant) followed by Ma-35 (2.10/plant) and Bb-45 (1.90/ plant) which were at par with each other as represented in Table 1. Similarly during 2016, it ranged from 3.38 to 5.35/plant compared to untreated control which recorded 6.78/plant with lower incidence of 3.38/plant in Bb-5a and Ma-35 treated plots (Table 2). The pooled data showed that the exit holes ranged from 2.59-4.48/plant compared to untreated control of 6.99/plant. The lower no. of exit holes were noticed in Bb-5a (2.59/plant) followed by Ma-35 and Bb-45 with 2.79 and 3.50/plant respectively and were at par with each other (Table 3). The decrease in exit holes in the present investigation may be because of lesser survival of *C. partellus* larva due to endophytic activity of *B. bassiana* and *M. anisopliae* isolates in the maize plant system. It has been reported that the endophytic EPF isolates are reported to produce secondary metabolites which cause feeding deterrence or antibiosis or leading to mortality or less infestation resulting in suppression effect on insect pests (Vestergaard *et al*. 2003). Conidial suspension of

Table 3 Pooled data of 2015 and 2016 on the effect of endophytic EPF isolates on maize stem borer

| Treatment | Isolate | Average No. of dead hearts | Dead heart (%) | No. of exit holes/plant | Stem tunneling/ plant (cm) | Cob yield/ 10 plants (kg) | Cob yield (tonnes/ha) |
|---|---|---|---|---|---|---|---|
| T1 | Bb-5a | $2.85^{a}$ (1.83) | 9.50 | $2.59^{a}$ (1.76) | $3.98^{a}$ (2.12) | $13.0^{a}$ (3.68) | 72.2 |
| T2 | Bb-23 | $5.13^{b}$ (2.37) | 17.08 | $4.48^{b}$ (2.23) | $6.47^{bc}$ (2.64) | $11.0^{ab}$ (3.39) | 61.1 |
| T3 | Bb-45 | $5.28^{bc}$ (2.40) | 17.58 | $3.50^{ab}$ (2.00) | $5.53^{ab}$ (2.46) | $11.9^{ab}$ (3.52) | 66.1 |
| T4 | Ma-35 | $3.81^{a}$ (2.08) | 12.71 | $2.79^{ab}$ (1.81) | $4.36^{ab}$ (2.20) | $12.6^{ab}$ (3.62) | 70.0 |
| T5 | Control | $6.41^{c}$ (2.63) | 21.38 | $6.99^{c}$ (2.74) | $8.01^{c}$ (2.92) | $10.9^{b}$ (3.37) | 60.3 |
| CD (P = 0.05) | | 1.2 | | 1.81 | 2.33 | 2.07 | |

Means followed by the similar letters in the columns are not significantly different at 5% by DMRT.

Bb-5a Control

Fig 1 Extent of stem tunneling caused by *C. partellus* in Bb-5a treated and untreated control plants.

endophytic *B. bassiana* and *M. anisopliae* sprayed on maize plants under greenhouse condition showed reduction in exit holes (0.2-3.3/plant) (Tefera and Pringle 2007).

*Cob yield*

The experimental results of pooled cob yield data revealed that all the four EPF isolates tested showed cob yield ranging from 72.2 to 61.1 tonnes/ha, however the maximum yields were noticed in Bb-5a (72.2 tonnes/ha) and Ma-35 (70.0 tonnes/ha) treated plots which are significantly higher than the untreated control (60.3 tonnes/ha) as depicted in Table 3. The higher yields obtained with these endophytic isolates may be due to effective endophytic suppressive of these isolates on maize stem borer. Reddy *et al*. (2009) reported significantly higher yield of 4.49 kg/20 plants in sorghum plots treated with endophytic *B. bassiana* compared to untreated control (2.84 kg) with artificial infestation of *C. partellus*.

Among the four endophytic EPF isolates tested, Bb-5a isolate of *B. bassiana* and Ma-35 isolate of *M. anisopliae* showed superior suppression of maize stem borer, *C. partellus* resulting in increased yield. This technology is eco-friendly and do not have any adverse affect on environment and human or animal health and hence can be recommended to farmers against maize stem borer as an alternative to chemicals insecticides.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India, for the constant support provided throughout the research.

REFERENCES

Anonymous. 2016. www.indiastat.com Ministry of Agriculture & Farmers Welfare, Government of India.

Bing L A and Lewis L C. 1991. Suppression of *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) by endophytic *Beauveria bassiana* (Balsamo) Vuillemin. *Environmental Entomology* **20**(4): 1207–11.

Cherry A J, Banito A, Djegui D and Lomer C. 2004. Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera: Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. *International Journal of Pest Management* **50:** 67–73.

Jalali S K and Sihgh S P. 2003. Bio-ecology of *Chilo partellus* (swinhoe) (Lepidoptera: pyralidae) and evaluation of its natural enemies - A review. *Agricultual Reviews* **24:** 79–100.

Jalali S K and Singh S P. 2002. Seasonal activity of stem borers and their natural enemies on fodder maize. *Entomon* **27**(2): 137–46.

Lewis L C, Berry E C, Obrycki J J and Bing L A. 1996. Aptness of insecticides (*Bacillus thuringiensis* and carbofuran) with endophytic *Beauveria bassiana* in suppressing larval population of European corn borer. *Agricultural Ecosystems and Environment* **57:** 27–34.

Lewis L C, Bruck D J and Gunnarson R D. 2002. On-farm evaluation of *Beauveria bassiana* for control of *Ostrinia nubilalis in* Iowa, USA. *Bio Control* **47:** 167–76.

Miranpuri G S and Kachatourian G G. 1993. Role of bioinsecticides in integrated pest management and insect resistance management. *Journal of Insect Science* **6:** 161–72.

Ramanujam B, Poornesha B, Yathish K R and Renuka S. 2015. Evaluation of pathogenicity of different isolates of *Metarhizium anisopliae* (Metchnikoff) Sorokin on maize stem borer *Chilo partellus* (Swinhoe) using laboratory bioassay method. *Biopesticides International*. **11**(2): 89-95.

Ramanujam B, Renuka S, Poornesha B and Shylesha A N 2016. Electron microscopic studies for confirmation of endophytic colonization of *Beauveria bassiana* in maize. *Journal of Pure and Applied Microbiology.* **10**(4): 3017–21.

Reddy N P, Khan A P A, Devi U K, Sharma H C and Reineke A. 2009. Treatment of millet crop plant (*Sorghum bicolor*) with the entomopathogenic fungus (*Beauveria bassiana*) to combat infestation by the stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae). *Journal of Asia-Pacific Entomology* **12:** 221–6.

Renuka S, Ramanujam B and Poornesha B. 2016. Endophytic ability of different isolates of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin in stem and leaf tissues of maize (*Zea mays* L.). *Indian Journal of Microbiology* **56:** 126–33.

Renuka S, Ramanujam B, and Poornesha B. 2015. Screening of *Beauveria bassiana* (Balsamo) Vuillemin isolates against maize stem borer, *Chilo partellus* (Lepidoptera: Pyralidae) and the effect of solid substrates on conidial production and virulence. *Journal of Pure and Applied Microbiology* **9:** 2979–86.

Sarup P, Siddiqui K H and Marwaha K K. 1987. Trends in maize pest management research in India together with bibliography. *Journal of Entomological Research* **11**(1): 19–68.

Tadele Tefera and Pringle K L. 2007. Biological control of the spotted stem borer *Chilo partellus* (Swinhole) (Lepidoptera: Crambidae) with the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. *Ethiopian Journal of Science* **30**(1): 65–70.

Vestergaard S, Cherry A, Keller S and Goettel M. 2003. Safety of hyphomycete fungi as microbial control agents. (*In*) *Environement Impacts of Microbial Insecticides,* pp 35-62. Hokkanen H M T and Hajek A E (Eds). Kluwer Academic Publishers, Dordrecht.