In-vitro study to determine the dose of *Beauveria bassiana* for management of *Spodoptera frugiperda*

ABSTRACT

Pest insects known as fall army worm (*Spodoptera frugiperda*) can feed on over 80 different types of crops. It has the potential to significantly reduce the yields of major cereal crops including oats (*Avena sativa* L.). The oat crop is one of the preferred hosts of this pest. This fodder crop is directly used as animal feed so, chemical control of pests is not preferable. One of the alternatives for the management of fall army worm on fodder oats is the use of microbials which have some advantages over the use of chemical insecticides, as they are highly host specific with low environmental contamination. *Beauveria bassiana* is a fungus and can be used to control this pest. So, the present study was undertaken to standardize the dose of *Beauveria bassiana* for management of fall army worm. The virulence test was conducted against third instar larvae of *S. frugiperda*. The larvae were treated with three doses of *B. bassiana* of 1×10^{12}spore/ml concentration *i.e.,*1, 2, and 3ml/L, by dipping them for 30 seconds in 10 ml of suspension. In case of control, larvae were treated with distilled water containing a drop of Tween- 80 (0.02%). At 24 hours after treatment, the differences in the mean larval mortality among different doses of *B. bassiana* were non-significant. However, at 48 and 72 hours after treatment, the differences in the mean larval mortality among different doses of *B. bassiana* were significant. Highest larval mortality was recorded at 48 and 72 hours after treatment in 3 ml/L (29.39 and 86.67%, respectively).

Keywords: Insect, Pest management, Cereal crop, Fungi, *Spodoptera frugiperda*

1. INTRODUCTION

"Oat (*Avena sativa* L.) is a cereal crop ranked sixth in the world cereal production, after wheat, rice, maize, barley and sorghum" (Devi et al.,2019). "Oat has assumed considerable importance in India as fodder as well as grain for animal feed particularly calves and young stock, horses, poultry and sheep. As a fodder crop, it has excellent protein quality, fat and mineral content. It is a palatable, succulent and nutritious crop" (Kapoor and Singh 2020). "Oat has adequate soluble carbohydrates, fibers and provides one of the richest sources of the dietary soluble fiber beta glucan, providing 5.0 g (oat meal) to 7.2 g (oat bran) per 100g serving" (Peterson et al.,2005). "In India, oat is grown as *rabi* season crop and has excellent growth habit with good quality herbage and recovers quickly after cutting. Oat requires a long and cool season for its growth. The total area, production and productivity of oats in the world during 2020-21 was about 10 million hectares with a production and productivity of 25.51 million metric tonnes and 2.55 metric tonnes per hectare, respectively" (USDA, 2022).

Various pests cause damage to oat plants throughout their life and no stage of the crop is free from damage. Pests of oats are either polyphagous or oligophagous and are attacked by several insect pests *viz.,* armyworm, cereal leaf beetle, cutworm, wheat aphid, plant bug, grasshopper, oat thrips, wireworm, cockchafer, fruit fly *etc*. Fifteen insect pests and natural enemies, belonging to 5 different orders have been recorded in oats (Kumar et al.,2017). Recently a new invasive pest, known as the fall army worm (FAW) (*Spodoptera frugiperda*J.E. Smith) (Lepidoptera: Noctuidae) has been introduced in India and it was first reported from College of Agriculture, University of Agriculture and Horticulture Science, Shivamogga, Karnataka (Sharanabasappa et al.,2018).Oat crop is one of the preferred hosts of this pest. Since this fodder crop is directly used as animal feed (An et al., 2020), chemical control of pest is not preferable and it is well known that some synthetic insecticides may deplete natural enemy population, cause contamination problems and most importantly, they are toxic to human and animals (Tabashnik et al.,2014). One of the alternatives for the management of FAW on fodder oats are the use of microbials which have some advantages over the use of chemical insecticides, as they are highly host specific and also induce low environmental contamination (Romero-Arenas et al., 2014). Among entomopathogenic fungi, *Beauveria bassiana*was found more toxic to *S. frugiperda*and caused 79% larval mortality (Shahzad et al.,2021; Fazlullah et al., 2023). Keeping in mind the above facts, the research work was carried out with the objective to standardize the dose of *Beauveria bassiana*for management of fall army worm.

2. MATERIALS AND METHODS

Media preparation
Potato dextrose agar (PDA) media was prepared for mass culturing of *B. bassiana*. About 250g of potato was washed, peeled, sliced into small pieces, to which 1000 ml distilled water, 20g agar and 20g dextrose was added and the mixture was cooked for 20-30 minutes. The potato extract thus obtained, was filtered through a muslin cloth. Thereafter, the media was poured into 250ml conical flask, plugged with nonabsorbent cotton and was covered with paper sheet and tied tightly with rubber band, and was autoclaved for sterilization at 15 lbs pressure and 121ºC for 15 minutes (Asi et al.,2013).

Culturing of *B. bassiana*
Pure mother culture of *B. bassiana* was obtained from Biocontrol Research Center and Production Centre, JNKVV, Jabalpur, which was multiplied and maintained on PDA slants at 4 ºC under refrigerated conditions for further use (Shah, 2018). The conidia were harvested by scraping the surface of old culture gently with inoculation needle and were inoculated on 1-2 days old PDA media filled Petri plates and kept in BOD at 26 ± 2 ºC temperature and 80±5% RH (Asi et al., 2013).

Preparation of fungal suspension
After 15 days of incubation, aqueous conidial suspensions (10 ml) were made from conidia harvested from the slants and Tween-80 (0.02%) was added to disperse the conidia and was filtered through a double layered muslin cloth. The number of conidia per ml was counted by using hemocytometer (Ramos et al.,2020) and fungal suspension of concentration of 1×10^{12}spores/ml was prepared.

Bioassay against *S. frugiperda* third instar larvae
"The virulence test was conducted against third instar larvae of *S. frugiperda* as per the methodology proposed by Moorthi et al.,(2011). The third instar larvae were treated with three doses of *B. bassiana* of 1×10^{12}spore/ml concentration *i.e.,*1, 2, and 3ml/L, by dipping them for 30 seconds in 10 ml of suspension. In case of control, larvae were treated with distilled water containing a drop of Tween- 80 (0.02%). Treated larvae were allowed to crawl freely on the blotting paper to remove excess moisture. Thereafter the larvae were placed individually in insect rearing box. All treated larvae were incubated at 27±1˚C, 65±5% relative humidity and photo phase of 14:10 hours. Fresh leaves of fodder oat genotype OS-6 were provided as food source for the larvae which were replaced regularly at an interval of 24 hours (Asi et al.,2013). Larval mortality was recorded daily and continued till complete mortality was attained or emergence of pupae/adult whichever was earlier". [25] The corrected mortality was calculated by using Abbott's formula (Prasad, 2014).
Corrected Mortality (CM) = (T – C/100 – C) × 100
Where,
T = Mortality in treatment (%)
C = Mortality in the control (%)

Statistical Analysis
Analysis of the different variables was carried out to know the degree of variation amongst all the treatments. The data was statistically analyzed by applying Factorial CRD and analysis of variance of different observations. The skeleton of ANOVA for Factorial CRD is presented as proposed by Sharma (2011).

3. RESULTS AND DISCUSSION
The current *In vitro* investigation assessed the management potential of *Beauveria bassiana* isolate against invasive FAW. Entomopathogenic fungi, for example *B. bassiana* are biocontrol agents with significant virulence characteristics that are quickly replacing synthetic insecticides (Idrees et al., 2023).

At 24 hours after treatment (HAT)

Data in Table 1 revealed that at 24 HAT, the differences in the mean larval mortality among different doses of *B. bassiana* were non-significant and it ranged from 2.72 (both in 1 and 2 ml/L) to 4.94% (3 ml/L). As there was no mortality in the control, hence the corrected mortality could not be computed.

At 48 and 72 HAT

At 48 and 72 HAT, the differences in the mean larval mortality among different doses of *B. bassiana*were significant. Highest larval mortality was recorded at 48 and 72 HAT in 3 ml/L (29.39 and 86.67%, respectively) and was followed by treatment 2 ml/L (27.17 and 73.33%, respectively) and lowest in 1 ml/L (22.72 and 73.33%, respectively). However, all the treatments were significantly superior to control at 48 and 72 HAT (4.94 and 6.66 %, respectively). Computation of corrected larval mortality at 48 and 72 HAT was minimum with 1 ml/L (23.67 and 69.05%), followed by 2 ml/L (19.07 and 76.19 %, respectively) and highest at 3 ml/L (26.06 and 76.19%, respectively).

At 96, 120 and 144 HAT

The result revealed that at 96 and 120 HAT, the differences in the mean larval mortality among different doses of *B. bassiana*were found to be significant. Maximum larval mortality was recorded at 96 and 120 HAT in 3ml/L (95.56 and 100%, respectively) and was followed by 2ml/L (93.33 and 97.44%, respectively) and minimum in 1ml/L (88.89 and 97.78 %, respectively). However, all the treatments at 96 and 120 HAT were significantly superior to control (11.11 and 13.33 %, respectively). The larval mortality recorded at 144 HAT was 100% in 2 ml/L and was followed by 1 ml/L (97.78%).Highest larval mortality (3rd instar larvae) was recorded with *B. bassiana*@ 3ml/L, and it was lowest in 1 ml/L, but was significantly superior than the control.

The present findings confirm the findings of Petlamul and Prasertsan (2012). The larval mortality was observed to increase with the increase in time, as is evident by and highest mortalities recorded at 24 and 144 hours after treatment (HAT). Similar findings have been reported by Pragya (2019). Maximum *S. frugiperda*larval mortality (100.00%) was observed with *B. bassiana*(1×1012 spores/ml) @ 3ml (1×1012 spores / ml) at 120 HAT. It can be inferred that larval mortality was dose and time dependent. The present findings corroborate the findings of Moorthi *et al.,* (2011), Pragya (2019) and Kumar *et al.,* (2021), as they also observed that the larval mortality increased both with the dose and time.Some *B. bassiana* isolates have been reported more pathogenic than others depending on the instar stage; for example, the *B. bassiana* isolates were shown to be more effective against early instar larvae than later instar lepidopteran larvae. An isolate of *B. bassiana* caused larval mortality against a second instar of FAW in one of the earlier investigations (Idrees *et al.,* 2022). *B. bassiana* also produces secondary metabolites that include a range of poisons such as beauvericin, bassianin, bassianolide and oxalic acid. These toxins enable it to parasitize and destroy its hosts (Wang *et al.,* 2021; Khamis *et al.,* 2023).

4. CONCLUSION

Beauveria bassiana isolate worked well against fall army worm while lowering the FAW population. The current investigation found that the maximum larval mortality was measured at 3 ml/L at 96 and 120 HAT (95.56 and 100%, respectively), followed by 2 ml/L at 93.33 and 97.44%, and the minimum at 1 ml/L (88.89 and 97.78 %, respectively). However, for the management of the FAW population, all therapies at 96 and 120 HAT were found to be considerably better than control (11.11 and 13.33%, respectively). Consequently, the isolate might offer a foundation for the creation of commercial biological pesticides. The primary toxins that influence the virulence factors of the entomopathogenic fungal isolate *Beauveria bassiana* require more investigation.

Table 1: Efficacy of *Beauveria bassiana*(1×1012 spores/ml) on *S. frugiperda*larvae (3rd instar) at different doses and hours after treatment

Dose (ml/L)	Mean mortality (M) and Corrected mortality (CM) of *S. frugiperda*larvae (%) at different HAT *											
	24		48		72		96		120		144	
	M#	CM	M*	CM	M*	CM	M*	CM	M*	CM	M*	CM
1.0	2.72 (7.88)	2.72 (7.88)	22.72 (22.11)	19.07 (19.67)	73.33 (57.52)	69.05 (56.23)	88.89 (70.73)	87.55 (69.53)	97.78 (85.01)	97.44 (84.63)	97.78 (85.01)	97.43 (84.63)
2.0	2.72 (7.88)	2.72 (7.88)	27.17 (31.30)	23.67 (28.89)	73.33 (61.93)	76.19 (60.85)	93.33 (77.87)	92.31 (76.93)	97.44 (84.63)	97.44 (84.63)	100 (90.00)	100 (90.00)
3.0	4.94 (11.70)	4.94 (11.70)	29.39 (32.80)	26.06 (30.65)	86.67 (62.25)	76.19 (61.17)	95.56 (82.86)	95.24 (82.60)	100 (90.00)	100 (90.00)	-	-
Control	0.5 (4.05)	-	4.94 (11.70)	-	6.66 (14.96)	-	11.11 (19.27)	-	13.33 (21.41)	-	13.33 (21.42)	-
SEm±	**4.68**	**5.41**	**4.41**	**4.87**	**3.20**	**3.88**	**7.09**	**8.42**	**4.99**	**6.20**	**-**	**-**
CD at 5%	**NS**	**NS**	**10.16**	**11.92**	**7.39**	**9.48**	**16.34**	**20.61**	**11.5**	**15.17**	**-**	**-**

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