THE EFFECTIVENESS OF INDIGENOUS ENTOMOPATHOGENIC FUNGI AS A BIOCONTROL AGENT OF *DORYSTHENES* SP. (CERAMBYCIDAE, COLEOPTERA) :

Identification, Biology, Efficacy and Its Epizootic

Pramono, Djoko* ; Aris Toharisman* ; Dendi Juliadi* ; Etik Mar'ati Achadian* and Dwi Purnomo Putranto**.

ABSTRACT

Boktor, *Dorysthenes* sp. (Cerambycidae, Coleoptera), is a new pest of sugarcane plantation in Indonesia. A serious study on its bioekology and behaviour as well as its integrated control including the use of entomopathogenic fungus just started in 1996. One of the promising indigenous fungi is coded name of J-12 isolate Subang.

The aim of this observation is to gain all basic information about the biology of this fungus, its efficacy under laboratory and semi field as well as field codition, mode of action and its symptom, and preliminary study on its epizootics in the sugarcane field.

This fungus was identified as *Metarrhizium flavoviride* Sorokin isolate Subang. Generally the result of all observation indicated that this fungi was quite effective and virulence to be developed as a biocontrol agent of boktor. Epizootics could be occurred in the field since one year after application. So therefore, formulation of this bioagent need to be observed in the near future.

Key Words : Sugarcane, boktor, *Metarrhizium flavoviride* Sorokin, biology, efficacy, epizootics, biological control.

*) Indonesian Sugar Research Institute (ISRI), Pasuruan, East Java – Indonesia.
**) Subang Sugarcane Estate, PT. Rajawali Nusantara Indonesia, West Java, Indonesia.

INTRODUCTION

Boktor was firstly reported by Rifal (1997) attacked on 8 hectars of Subang sugarcane estate in 1989, and then it became 100 hectars in 1995 (Pramono, 1997). A serious field observation conducted on May to July 1999 showed that all of the Subang sugarcane estate area (± 6.000 hectars) were infested by boktor on different infestation level (Pramono, *et al*., 1999). Recently, boktor has already infested a nearby sugarcane estate such as Jatitujuh.. The presence of boktor as a new sugarcane pest must be taken into serious consideration, otherwise it will be a very potential problem for Indonesian sugar industry in the future (Pramono, *et al*., 2000).

Larvae of boktor attack seed cane, young cane as well as mature cane. Usually the infestation of boktor in the field will be followed by infestation of termites, ants or some pathogens. So, it will destroy cane productivity and become an extra problem on cane juice processing in the factory. Besides, infestation of boktor on ratoon cane are more serious than on plant cane (Pramono, *et al*., 2000).

A serious IPM programm were studied since 1996 to combat boktor. One of the control measures using entomopathogenic fungus as a biological control agent was also observed. The code name fungus of J-12 is promising to be developed as an effective bioagent for controlling boktor. Inventory of the fungus had been done locally as the starting procedure and then was followed by pathogenicity tests as the first selection in the laboratory (Pramono, *et al*, 1999), culture, phytotoxicity test (Pramono, 1999), identification, efficacy under laboratory and glass house as well as field condition, and formulate the bioagent and its effectivity test.

According to the field survay conducted in 1999 showed that J-12 could only be found 0,67 % of the Subang sugarcane area (Pramono, Rifal and Putranto; 1999). This data will play an important role in evaluating the epizootic of the fungus in the near future.

MATERIALS AND METHODS

Identification and Description

The entomopathogenic fungus with J-12 (Subang isolates) was growth on Potato dextrose agar (PDA) and Miura medium (LCA). Identification was done by microscopic as well as macroscopic procedure. Shape and colour of hypha, conidia, conidiogenous cell and phialide were observed under microscope, while macroscopic observation tends to shape and colour of colony, growth of synnema and appearance of the fungus on its hosts. The key of identification follow the procedure of entomopathogenic fungal identification by Humber (1998).

Efficacy under Laboratory and Glass House

Randomized complete design with 5 treatments of the transport media and 10 replication were used for testing under laboratory and glass house condition. The transport media were respectively : (i) sterilized soil, (ii) sterilized soil + J12 spores, (iii) soil, (iv) soil + J12 spores, and (v) sterilized soil + powder of mummified larvae. Four kinds of instar larvae (2nd, 4th, 6th, and 8th) were put in each treatment. Observation in the laboratory had been done routinely until 1 month with the interval of 3 days, while under glass house condition weekly observation until 3 weeks. Number of mummified larvae were counted.

The influence of spores concentration on the survival of larvae had been tested under glass house condition using randomised complete design with 5 treatments and 4 replication. The treatments were respectively : (i) sterilized soil as check plot, (ii) $94x10^3$ spores/ml, $94x10^4$ spores/ml, $94x10^5$ spores/ml, and $94x10^6$ spore/ml suspension. The other procedures of the observation was similar with the previous glass house observation.

Field Release Trial

The trials had been done in 1996/1997 and 1998/1999 at 3 plant cane (PC) area such as : Tanjungan 15 (2 Ha), Kumendung 58 and 59 (8 Ha), and Kumendung 50 (4Ha). Each area was divided into treatment plot and check plot.

The transport media with concentration of $94x10^6$ spores/ml suspension of J12 were distributed in the row of the treatment plots. The dose of the transport media was approximately 74 kg/Ha, while the application time was coinsiding with the seed cane planting time.

The observations on Kumendung 50, 58 and 59 were conducted before cultivation and during harvesting period by digging out the cane plant of 10 rows @ 10 m length. The parameters to be observed are respectively : (i) number of boktor population, and (ii) number of mummified boktor caused by infection of entomopathogenic fungi. While observation on Tanjungan 15 were conducted monthly by digging out cane plant of 4 x 2 rows @ 10 m length for observing the epizootics of *M. flavoviride* during one planting period.

RESULTS AND DISCUSSIONS

Identification and Description

According to Humber's key indicates that fungi with code J-12 seems like *Metarrhizium flavoviride* Sorokin. It based on conidia characters, colour of conidia and phialide (conidiogenous cell).

Hypha, conidia and other fungal structure visible on the exterior of the host. Hypha has no colour (hyaline), 2 – 3 μm wide. Conidia are formed on mycelium, while synnemata on the host body, no spores formed inside host cadaver. Conidia aseptate, cylindrical to ovoid, 7.52 – 8.78 μ m long, forming chains sometimes aggregate into prismatic columns, green or gray green. Conidiogenous cells conical apices, borne at apices of broadly branched (Figure 1).

Symptoms of the infected larvae is languid, cease to feed, reduce movement ability and finally it is dead. The dead body is sluggish, no smelled, and the become harden (mummified). White mycelium wholly covering its host in a few days. Furthermore the green spores appear from the mycelium in 2 – 3 days. The last attack showed that spores of fungus obscured insect body. The spores are green or gray green, dry and drops. At this stage, the insect's cadaver becomes dry and easy to be broken.

At the late stage of symptom, a macroscopic structure (synnema) projects from the host body. The synnema likes white trumpet with yellow compact mass on the basal and cottony mass on the apical (Figure 2). The macroscopic structure appears from both insect cadaver and the fungi that growth on the PDA medium.

Efficacy under Laboratory and Glass House

The complete result of the laboratory observation is presented in Figure 3 to 6. Prinsipally all instar larvae can be infested by the fungus in all treatments, except check plots. The differences between treatment just the time and number of infested larvae.

According to those figures, it can be conclude that (i) an older instar larvae is more susceptible to the fungus than the younger instar, (2) a smaller particle of the transport media formulation have a better opportunity to distribute in the soil and will resulting a better chance to make a contact with its host. Based on those results indicate that the improvement of the bioagent formulation and constructing its field applicator unit play an important role in the success of applying biological control program in a large scale at the future.

In general the result of the experiments show that *M. flavoviride* Sorokin indicated as an effective biotic agent for controlling boktor. Besides, the effectiveness of its application depend on the transport media being used and the instar larvae as a target insect.

The results of the observation under glass house condition are mentioned on Table 1, while the effect of the spores concentrations on the time and number of infested larvae are mentioned on Table 2. Accordingly, it can be assumed that spores concentration affecting the survival of the host larvae. The time to kill 50% of the treated larvae (LT_{50}) ranged from less than 2 weeks (94 x 10^6 spores/ml) up to 3 weeks (94 x 10^3 spores/ml). Those data are similar with the result of the observation on concentration of *M. anisopliae* spores and its effect to *Adoryphorus couloni* (Rath and Worledge, 1995).

Table 1. Efficacy of different transport media of *M. flavoviride* on the different instar larvae under glass house condition.

Instar larvae	*Transport media*	*Number of infested larvae (%).*		
		1st week	*2nd week*	*3rd week*
8th instar	Sterilized soil	0	0	0
	Sterilized soil + spores	0	30	30
	Original soil	10	60	80
	Original soil + spores	0	90 *)	100
	Sterilized soil + powder of the mummified larvae.	20	80	90
6th instar	Sterilized soil	0	0	0
	Sterilized soil + spores	10	30	30
	Original soil	0	60	90
	Original soil + spores	20	90 *)	90
	Sterilized soil + powder of the mummified larvae.	0	40	90
4th instar	Sterilized soil	0	0	0
	Sterilized soil + spores	0	10	30
	Original soil	10	80	90
	Original soil + spores	60	100 *)	100
	Sterilized soil + powder of the mummified larvae.	10	30	60
2nd instar	Sterilized soil	0	0	0
	Sterilized soil + spores	0	20	20
	Original soil	70	80	90
	Original soil + spores	70	90 *)	100
	Sterilized soil + powder of the mummified larvae.	10	60	70

Table 2. The influence of the spore concentration in the transport media on the time and number of infested larvae.

No.	***Concentration of spores/ml***	***Number of larvae infested (%) After aplication***				
		1 week	***2 weeks***	***3 weeks***	***4 weeks***	***5 weeks***
1.	**94 x 10^3 spores**	18,75	43,75	50	56,25	62,50
2.	**94 x 10^4 spores**	0	31,25	68,75	68,75	68,75
3.	**94 x 10^5 spores**	18,75	81,25	81,25	81,25	81,25
4.	**94 x 10^6 spores**	18,75	93,75	93,75	93,75	93,75
5.	**Check plot**	6,25	12,50	12,5	31,25	37,50

Field Release Trial

The result of the field trials show that the biological control plots are different compared to the check plot. The comparison parameters are respectively (i) population of boktor, and (ii) the level of boktor infected by *M. flavoviride* Sorokin.

The observations show that the number of boktor population in treatment plots is approximately 34 % lower than check plot in harvesting period. The data means that the biological control approach has a high contribution in decreasing boktor population in the field. While the level of larvae infected by *M. flavoviride* Sorokin in the treatment plots still low in number (15,33% - 17 %) eventhough higher than the check plot. This data just only came from the harvesting period of the plant cane (PC) or 1 year after the application of bioagent formulation. Samuels *et al*.(1990) reported that a strong growth responses exhibited in the treated plots of *M. anisopliae* after the third ratoon cane in Australia. In Reunion, the results revealed that *Beauveria brongniartii* was present more than 3 years and gradually reduced the grub populations under an economic threshold with only one application (Goebel and Vercambre, 1995).

Special monthly observation on the epizootic of *M. flavoviride* Sorokin during a year of planting period (PC) in Tanjungan 15 is mentioned on figure 7. According to this figure indicates that *M. flavoviride* Sorokin is promising to develop by themselves on the field in the future. Hopefully the epizootic will occurr even in the ratoon cane in which the control measures of boktor is usually difficult to be done. Vercambre *et al*.(1994 and 1996) reported that application of *Beauveria brongniartii* on plant cane could control the white grub *H. marginalis* until 6 years planting period in Reunion Island.

CONCLUSION

According to the result of identification indicates that the indigenous entomopathogenic fungi with code J-12 is belong to *Metarrhizium flavoviride* Sorokin. The symptoms of the infected larvae is languid, cease to feed, reduce movement ability and finally it is dead. The dead body is sluggish, no smelled, become harden (mummified), whooly covering by green spores. At the late stage of symptom, a macroscopic structure (synnema) projects from the host body.

Efficacy under laboratories and glass houses condition conclude that the effectiveness of *M. flavoviride* depend on the form and size of the transport media formulation being used and the instar larvae of boktor as a target pest.

The field release trials shows that *M. flavoviride* reducing 34 % of boktor population and increasing the fugus infestation 0f 15,33 % - 17 % in the treatment plots at harvest. Monthly observation during plant cane (PC) period indicate that the epizootic process of *M. flavoviride* have been occurred in the treatment plots eventhough still in a low number. Hopefully the epizootic will occurred much higher in the ratoon cane (R).

REFERENCES

Goebel, R. and B. Vercambre. (1995). Use and development of an entomopathogenic fungus to control *Hoplochelus marginalis* (Col : Scarabaeidae), a major pest of sugarcane in Reunion Island. The XIII International plant protection congress. The Hague-The Netherlands, 2-7 July 1995. European Journal of Plant Pathology. Khuver Academic Publishers. 594 – 600.

Humber, Richard A. (1998). Entomopathogenic fungal identification. APS/ESA Workshop. November 7, 1998. APS/ESA joint annual meeting, Las Vegas, NV. 26 pp.

Poinar, George O. Jr. and Gerard M. Thomas. (1984). Laboratory guide to insect pathogens and parasites. Plenum Press. New York and London. 105 – 169.

Pramono, D. (1999). Pathogenicity and phytotoxicity test of the entomopathogenic fungi of J-12. ISRI, Pasuruan. 11 pp. (*English summary*).

Pramono, D.; Etik M. Achadian ; Murwandono and Abdul Rifal. (1999). Inventory and selection of entomopathogenic fungi of the white grub and cerambycid larvae on the sugarcane plantation. ISRI, Pasuruan. 9 pp. (*English summary*).

Pramono, D.; Abdul Rifal and Dwi P. Putranto. (1999). Distribution and intensity of infestation of Dorysthenes sp. (Cerambycidae, Coleoptera) at Subang sugarcane estate-PT. RNI II Cirebon. ISRI, Pasuruan. 10 pp. (*English summary*).

Pramono, D.; Abdul Rifal; Dendi Juliadi and Etik M. Achadian. (2000). Boktor (Cerambycidae, Coleoptera), hama potensial baru pada perkebunan tebu di Indonesia. Prosiding pertemuan teknis. Surabaya, 15-16 November 2000. ISRI Pasuruan. 9 pp. (*Indonesian language*).

Pramono, D.; Abdul Rival and Dwi P. Putranto. (2000). *Dorysthenes* sp. (Cerambycidae, Coleoptera), a new potential pest of sugarcane plantation in Indonesia : The biology and its integrated control. Paper submitted to the 24th ISSCT Congress, 16-21 September 2001, Brisbane, Australia. 13 pp.

Rath, A. C. and D. Worledge. (1995). The effect of *Metarhizium anisopliae* (DAT F-001) concentration and exposure time on the survival of the Subterranean pasture pest, *Adoryphorus couloni* (Col.: Scarabaeidae). Entomophaga 40 (1) : 35 – 45.

Samuels, K. D. Z.; D. E. Pinnock and R. M. Bull. (1990). Scarabeid larvae control in sugarcane using M. anisopliae. Journal of invertebrate pathology. 55 : 135 –137.

Vercambre, B.; O. Goebel; Guy Riba; Michel Marchal; Cecile Neuveglise and Pierre Ferron. (1994). Success in microbial control of a soil pest, Haplochelus marginalis, in Reunion Island : Choice of suitable fungus. Proc. of XXVII th annual meeting of the society for invertebrata pathology. Montpellier, France. 283 – 288.

Vercambre, B. (1996). Biological control against sugarcane white grub in Indonesia. Current situation and outlook. Draft report. CIRAD/CA Montpellier , France. 34 pp.

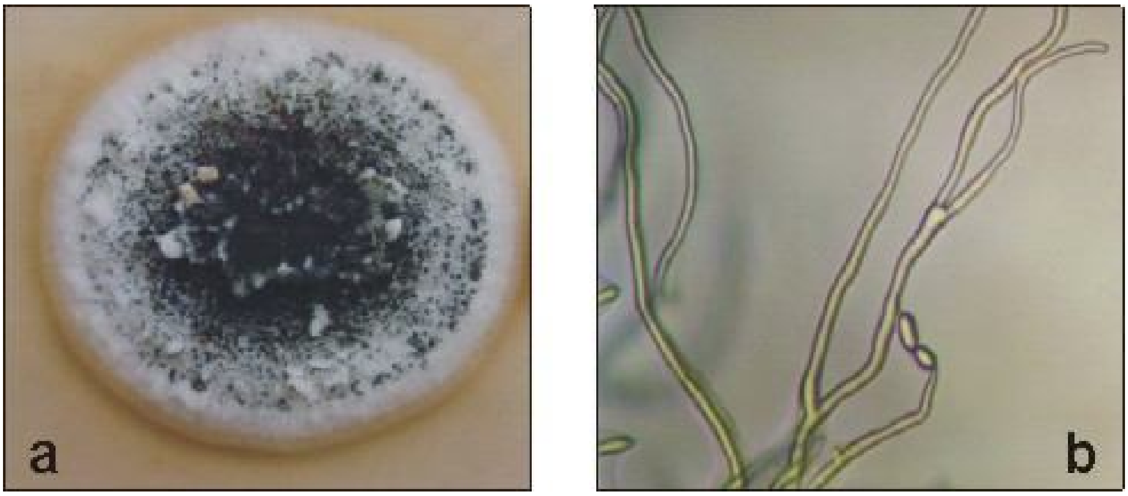

Figure 1. *Metarrhizium flavoviride* Sorokin (a) macroscopic structure on PDA medium, and (b) microscopic structure (hyfa and conidia).

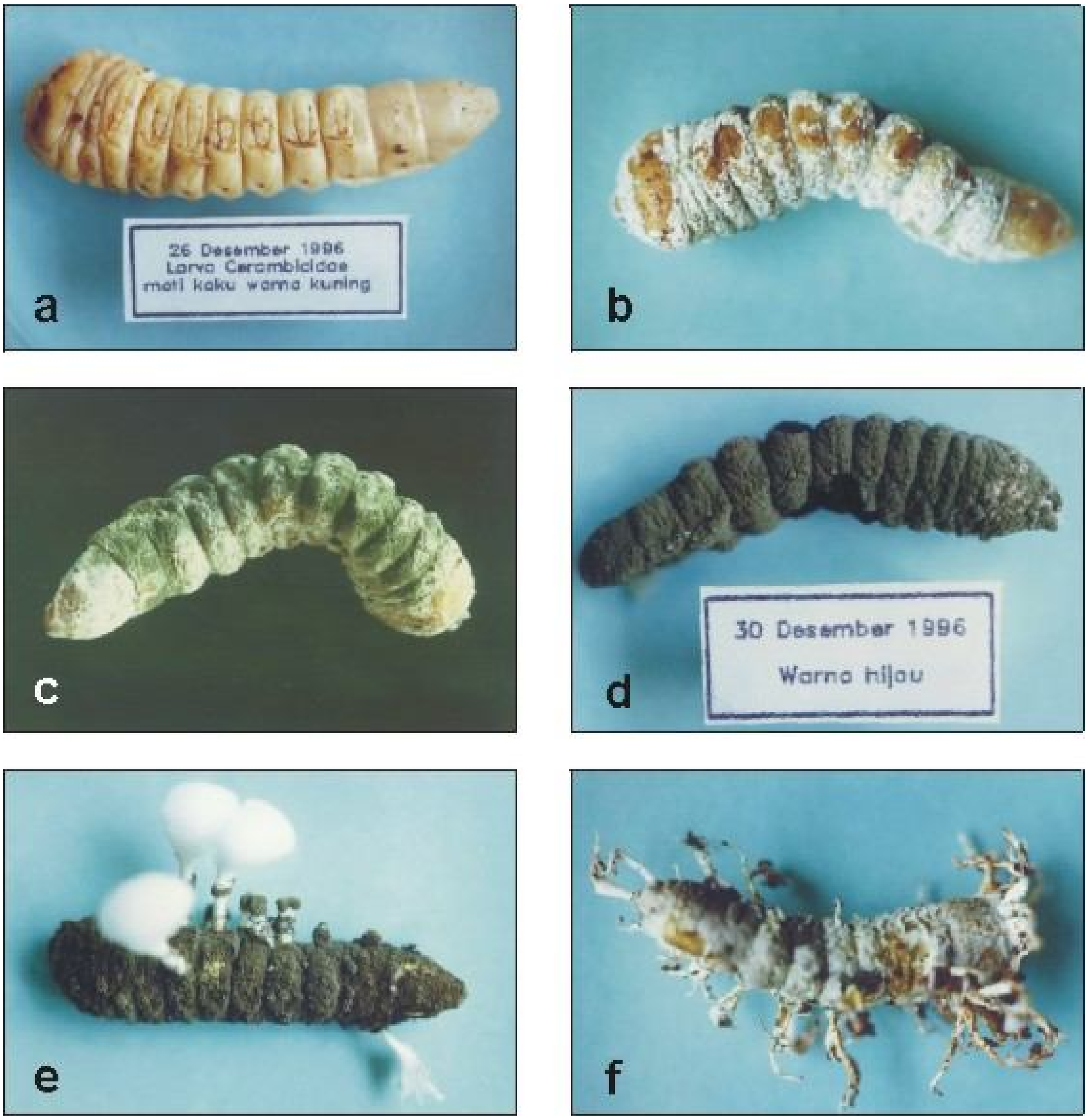

Figure 2. The typical symptom of *Dorysthenes* sp. larvae infected by *M. flavoviride* Sorokin.

***Note for figure 3 to 6* :**

- **A** = Sterilized soil.
- **B** = Sterilized soil + spores of *M. flavoviride* Sorokin.
- **C** = Original soil.
- **D** = Original soil + spores of *M. flavoviride* Sorokin.
- **E** = Sterilized soil + powder of mummified larvae

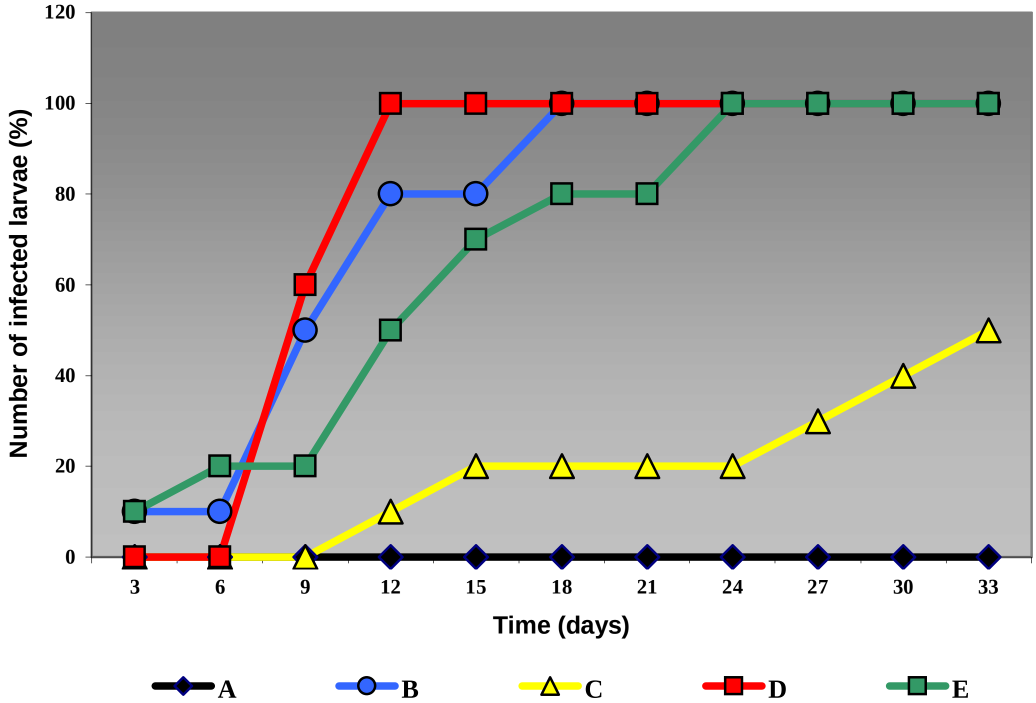

Figure 3. The influence of different transport media of *M. flavoviride* on the 8th instar larvae under laboratory condition.

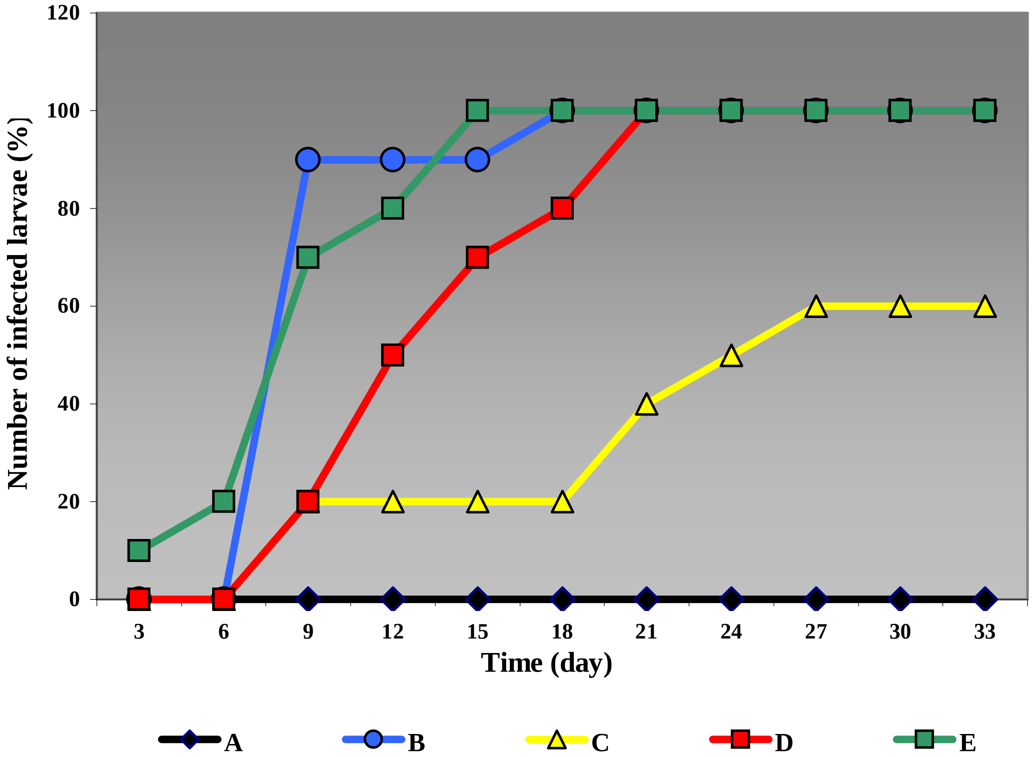

Figure 4. The influence of different transport media of *M. flavoviride* on the 6th instar larvae under laboratory condition.

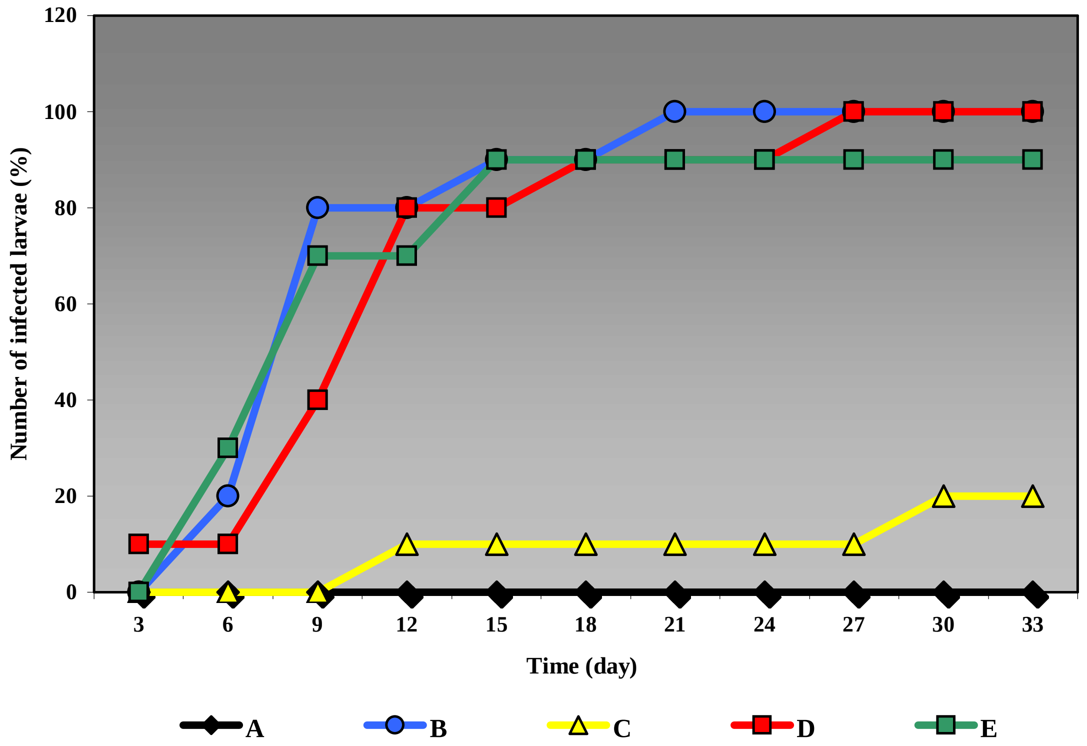

Figure 5. The influence of different transport media of *M. flavoviride* on the 4th instar larvae under laboratory condition.

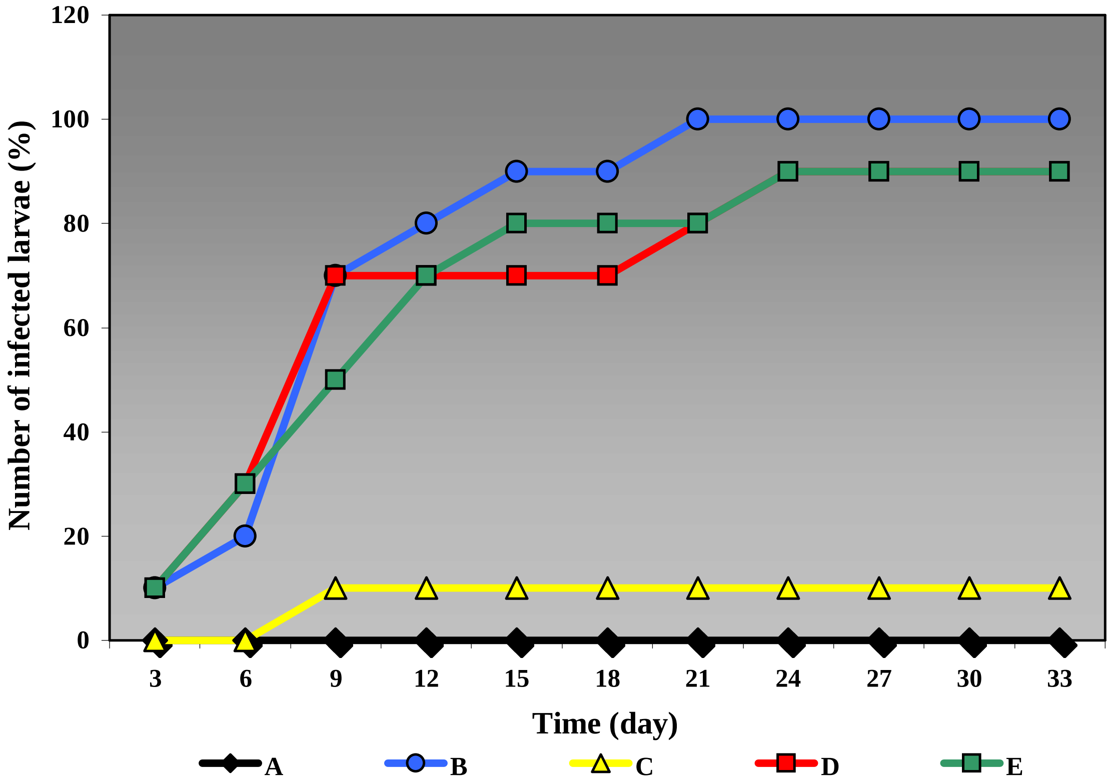

Figure 6. The influence of different transport media of *M. flavoviride* on the 2nd instar larvae under laboratory condition.

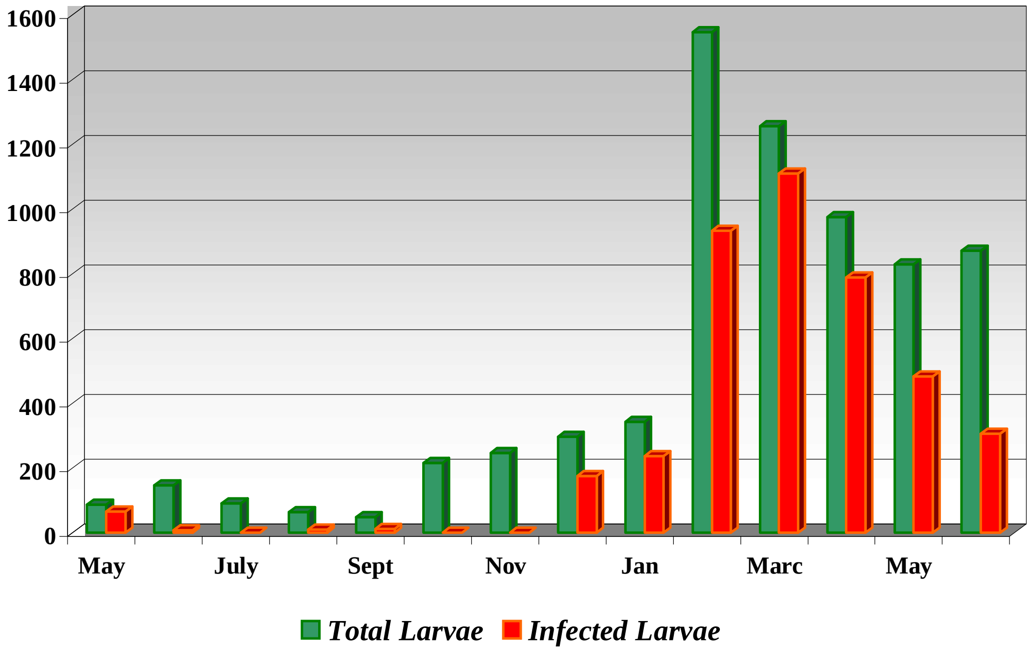

Figure 7. The epizootics process of the *M. flavoviride* Sorokin occurred in Tanjungan 15 during 1996/1997 planting period (PC).