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Research Article

ISSN: 2395 -5775

DEVELOPMENT OF AN INTEGRATED MANAGEMENT SYSTEM DISEASES CACAO *(THEOBROMA CACAO L.)* WITH EMPHASIS ON BIOLOGICAL CONTROL

Sebastián Yánez[1]., Bernardo Castro[2]., Leticia Silvestre[3]., Herman Hernandez[4] and Valdano Tafur[5]

Abstract

Within the project "Development of an integrated management of diseases of cacao with emphasis onbiological control", it aims to determine the level of optimization for mulations combining biocontrol agents for the management ofdiseases of cocoa. Both *Moniliophthorararoreri* (Cif and Par) as *Moniliophthorapernicious* (Aime andPhillips-Mora) are important pathogens that caused etriment to the productivity of cacao. Afriendly alternative environment is the use of Biological Control Agents (BCA). The evaluations were conducted in two seasons (June and January). Was used pods (about three months) both Trinitarian (ICS – 96 and CCN 51) as National (EET – 233 and EET-103). Plant antagonists were (*Trichodermakoningiopsis* 4.09 and *T.ovalisporum* 70a). Asinoculumvehicle were used five types of vegetable oil (corn, soybean, palm, sunflower and agricultural). Significant differences for the treatments were presented. The highest percentages of colonization in the early days of assessment were recorded for the treatment ICS -96 +*Tk* 4.09+agricultural oil with 86% (epiphytic) and ICS-96 +*Tk* 4.09+ Sunflower oil with 88% (endophytic). The lowest percentages were recorded for EET- 233 +*To* 70a+18% Oil agricultural and EET-233 +*To* 70a+Sunflower oil with 6%, epiphytic and endophytic respectively action. In the second epoch of the highest expressions evaluation presented them both in epiphytic 100% and endophytic 96% is treatment ICS – 96 + *To* 70a+soybean oil; the lowest percentages were recorded for EET– 233 + *To*70a+Soybean oil with 8% (epiphytic) and EET– 233 + *To*70a+Sunflower oil with 0% (endophytic). The plant material factor(Trinitarian and National) has high significance in the expression of either in oculum BCA vehicle.

Key Words: Pathogens, *Trichoderma* spp., Biological Control Agents, Emulsions

INTRODUCTION

Proper management of disease incacao (*Theobroma cacao* L.) can be considered to lower the height of the tree by a severe cut of tree, sanitary pruningand removal of diseased pods. Chemical controlis doneby protecting fruit with copper-based fungicides which reduces infections by 40%. The use ofnew alternatives such asthe introductionof antagonistic microorganisms *Moniliophthorararoreri* (Cif and Par) and *Moniliophthora pernicious* (Aime and Phillips-Mora), are a friendly alternative to the environment while also reducing the pathogen's ability to produce inoculum(Durango,2001). Both *M.roreri* and *M.pernicious*, for long periods oftheir life cycle, growing a symptomatically and intercellular inmeristematic tissues of cocoa; any endophytic can pre-colonize these potential areas of infection, which could potentially prevent the invasion of these pathogens through exclusion (competition), mycoparasitism, antibiosis and induced resistance. All these mechanisms have been reported for*Trichoderma* endophytes of cocoa (Bailey *etal.,* 2006; 2008, Mejia *et al.,* 2008). Early detection and intervention is crucial for profitable control of M.rorerias to maintain suitable conditions within a week you can see clear symptoms, the pathogens porulates prolifically on the surface of the ear (Krauss *etal.,* 2006).

Biological Control Agents (BCA), could improve drastically, timely disease, at a stage where the systemic action of the pathogen is not only relatively inaccessible, but also nearly impossible to diagnose in the field. The applications of BCA, in combination, cocoa plantations, for disease management are more efficient when they are used individually (Elad, 2003).

Apply BCA formulations with high potential for efficacy in cocoa plantations, leading to better manage diseases when applied individually (Castro and Yánez, 2013).

Today BCA applications incocoa are madeonce or twicea month. It is not clear what happens in the time periods since the continuous formation of new ears which may be unprotected. The devise mechanisms that can prolong and increase the epiphytic activity (epicarp) and endophytic (mesocarp) of the BCA undoubtedly help improve efficiency in the control of plant diseases. The impact of the formulation on the endophytic colonization of cacao has not been studied in detail. A suitable formulation can ensure rapid germination of spores and protection of the BCA (inoculate type) during the establishment of the plantation. Much research has been conducted with mycoherbicides detailing the benefits of optimization formulations. In efforts to improve the action of fungal bio her bicides, researchers have used hydrophilic polymergels (Shabana *etal.,* 1997) organo silicon surfactant (Zidack and Backman, 1996), oilinvert emulsions (Womack *etal.,*1996), nutrients (Boyette *et al.,* 1996) and other additives (Bailey *etal.,* 2004). The most effective formulations may be specific to microorganisms and tritrophic systems relations under study (with Host -Pathogen-Environment).
Within the scope of this investigation is to determine the level of optimization formulations combining bio control agents for the management of diseases of cocoa which evaluations are presented.

MATERIALS AND METHODS

The present investigation was conducted in facilities and experimental plots of the National Plant Protection

[1,4,5]Faculty of Agricultural Sciences. Central University of Ecuador. Quito – Ecuador
[2]PLANTABAL.Quevedo – Ecuador
[3]Entomology laboratory. Austral University of Chile. Valdivia – Chile
Correspondence and Reprint Requests: Sebastián Yánez
Received: October, 19 2017 | Accepted: November, 20 2017 | Published Online: December, 28 2017

Conflict of interest: None declared | Source of funding: Nil

Department (DNPV) Experimental Tropical Pichilingüe Station (EETP), the National Institute for Agricultural Research (INIAP) located in the Province of rivers Km5 track Quevedo-Empalme, located at the geographical coordinates 05 'South Latitude1°79°26' West Longitude. In experimental plots called"Local Clones", "Lot 233"and "Lot H1".

For the respective evaluations regarding the cocoa plant material pods (about three months) both Trinitarian(ICS – 96 and CCN51) as National (EET – 233 and EET-103) was used. The research was conducted at the field level in the first phase and the second under controlled conditions in the laboratory (*in vitro*). For both phases consisted of inoculation (manual spray) formulation (oil invert emulsions) with native isolates agents of bank collection that is in the DNPV - EETP (*Trichoderma koningiopsis* 4.09 and *T. ovalisporum* 70a). Production of *Trichoderma* were performed on the substrate grain rice *Oryza sativa* L., collecting spores with MYCOHARVESTER. The spores were stored at 4 ° C until use. For prepare emulsions mothers were used: water (500mL), lecithin(12.5 mL) and vegetable oil(487.5 mL). The inoculums concentration was obtained by counting in Neubauer®chamber, adjusting it to1 $x10^8$UFC.mL^{-1}. The combination of different factors under study determined the number of treatments (Table1).

Biological Response Window

Preliminary experiments were performed using combination of BCA; were handled strains of *Trichoderma* DIS 219b; DIS 70a; 4.09 *T.koningiopsis* Samuels, Smith & Evans and *T. stromaticum* Samuels & Pardo-Schulthei. These strains were combined in water without surfactant. SILWET® L and BREAK THRU® 100 SL for *Trichoderma*.

The mixture was kept at 25 °C for 24 hours. After dilutions were performed on plates with selective medium Corn Meal Dextrose Agar (CMDA) plus antibiotic (Chloramphenicol) 0.05% and colony-forming units (CFU) were counted.

Similarly germination tests were performed in a moist chamber simulators (100%), in which the emulsion was placed on glass slides containing sterile PDA®, at temperatures of 28 $\pm$ 2 °C; simulating field conditions, after which the spore germination and germ tube measurement was recorded.

Percentage Colonization

Trichoderma populations established on the pods were evaluated in twointervalsduring the experiment: a three and thirty days after application. The earswere washed with saponin-based solution, rinsed with distilled water before sectioning and take 5 discs(punched) by pod(epiphytic and endophytic). To then place exposed to nutrient medium.

Influence of Climatic Factors in the Persistence of BCA

Two trials were performed at different times of the year(June and January); with different plant materials but similarin nature (Trinitarian and National). Temperature, relative humidity, precipitation and heliophany: Daily climate data were recorded. With which the persistence of *T.ovalisporum*(To) and *T.koninguiopsis*(Tk) was determined over time, depending on type inundative- inoculative BCA. For the test two (January), at different time of the year the same treatments with the difference in plant material (CCN - 51 and EET-103) were used.

Table 1 Treatmentsin assessing thedevelopment of asystem of comprehensivedisease managementofcacao (*Theobroma cacao* L.) with emphasis onbiologicalcontrol.

TREAT.	DESCRIPTION (Formulations of BCA)
t1	ICS – 96 + *T. koningiopsis* 4,09 + Corn oil
t2	ICS – 96 + *T. koningiopsis* 4,09 + Soybean oil
t3	ICS – 96 + *T. koningiopsis* 4,09 + Palm oil
t4	ICS – 96 + *T. koningiopsis* 4,09 + Sunflower oil
t5	ICS – 96 + *T. koningiopsis* 4,09 + Agricultural oil
t6	ICS – 96 + *T. ovalisporum* 70a + Corn oil
t7	ICS – 96 + *T. ovalisporum* 70a + Soybean oil
t8	ICS – 96 + *T. ovalisporum* 70a + Palm oil
t9	ICS – 96 + *T. ovalisporum* 70a + Sunflower oil
t10	ICS – 96 + *T. ovalisporum* 70a + Agricultural oil
t11	EET – 233 + *T. koningiopsis* 4,09 + Corn oil
t12	EET – 233 + *T. koningiopsis* 4,09 + Soybean oil
t13	EET – 233 + *T. koningiopsis* 4,09 + Palm oil
t14	EET – 233 + *T. koningiopsis* 4,09 + Sunflower oil
t15	EET – 233 + *T. koningiopsis* 4,09 + Agricultural oil
t16	EET – 233 + *T. ovalisporum* 70a + Corn oil
t17	EET – 233 + *T. ovalisporum* 70a + Soybean oil
t18	EET – 233 + *T. ovalisporum* 70a + Palm oil
t19	EET – 233 + *T. ovalisporum* 70a + Sunflower oil
t20	EET – 233 + *T. ovalisporum* 70a + Agricultural oil

Statistical Analysis

The resultswere processed byANOVAfor differences between treatments, time and type of action (epiphytically and endophytically). In the case where significant differences multiple range tests LSD Fisher significance ($P < 0.05$) was performed; the Info Statstatistical program was used as an analysis tool.

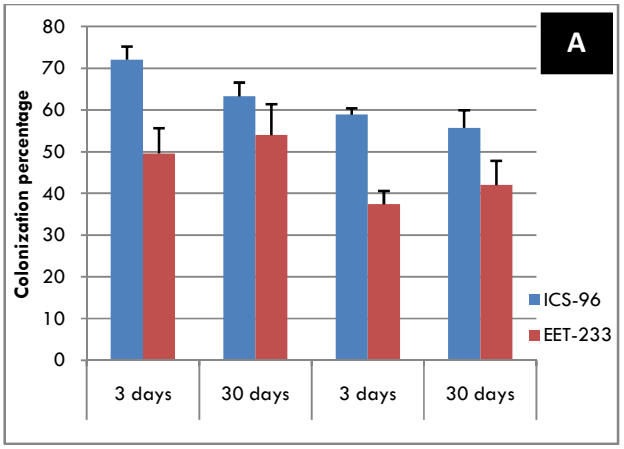
Path between the extents of colonization over time was assessed using regression analysis taking the time as independent variable; modelswere selected based on the best values ofR^2.

RESULTS AND DISSCUSION

Percentage Colonization

When comparing plant materials, regardless of the species of microorganism (*Trichoderma*) colonizing; you can clearly see that theTrinity-origin have greaterimpact on the colonization rate of National type (Figure1).

Similarly we can see that the epiphytic action is more marked than in the evaluation endophytic over time. Ascited Evans *et al.,* 2003a, by showing that To (TK-1) is an endophyte body, a fungus that develops asymptomatic infections in healthy plants to form a mutualistic symbiosis.



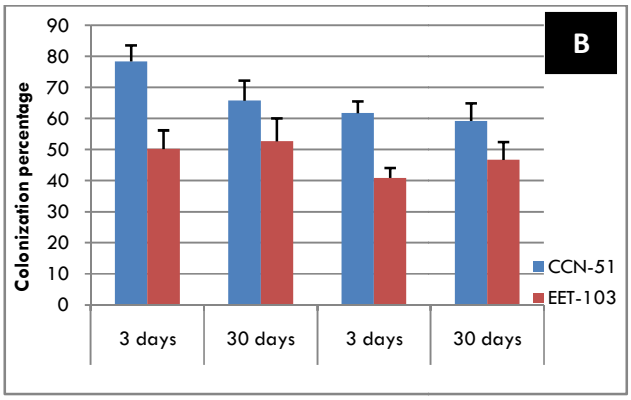


Figure 1 Percentage of BCA colonization by genetic (Trinitarian and National) of plant material in cacao (*Theobroma cacao* L.) in two seasons (A: June B:January).

Feature that backs up the results to be dormant after the body of the evaluation period, which leads toa preventive protection against pathogens incocoapod. Arnold and Herre (2003) demonstrated the potential of cocoa endophytes to protect seedlings from infection by *Phytophthora* sp. sheets. One argument in favor of BCA endophytes is that these face a wider window of opportunity for antagonism, since *M. roreri* spores germinate and penetrate the surface of the sheath shortly after landing there and establish a systemic infection it may lie dormant for 2 months (Evans, 1981).

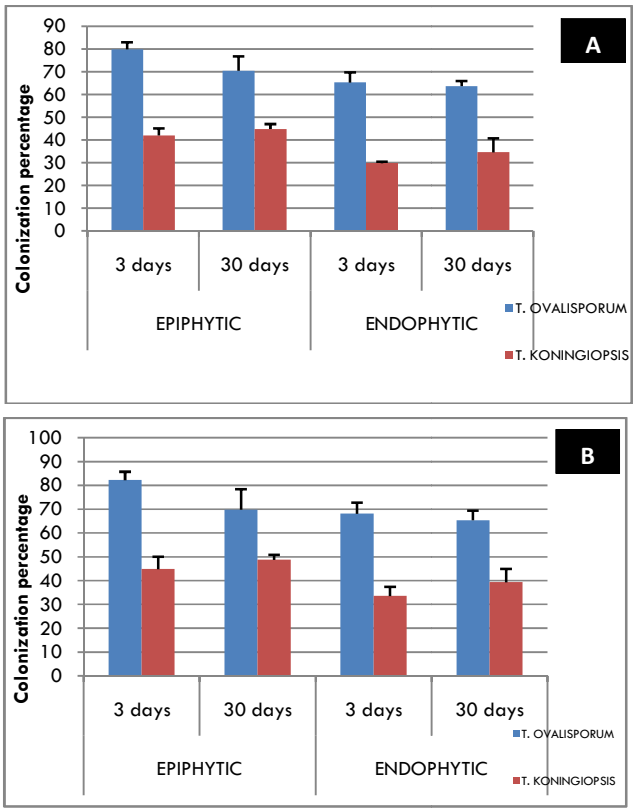


Figure 2 Percentage of BCA colonization influenced by the species of organism (*Trichoderma*) in different plant materials cacao (Trinitarian and National) in two seasons (A: June B:January).

Harman *et al.* (2000, 2004), detailed the ability of *Trichoderma* to aggressively to colonize plant roots, hence To show the fact that wide acceptance for colonization of stems, flower cushions and cocoa pods. In Trinitarian cocoa colonization percentage is greater than 50% constant regardless of the time of evaluation.

Regarding the percentage of colonization by the type of organism, *To* and *Tk*, can be seen (Figure 2) that either the time of year *To* have greater ability to colonize that *Tk*; this is what can be attributed to particular germ in their structures. You can see a decrease over time in the colonizing ability by *To*, both epiphytic and endophytic; *Tk* if we see that increasing the variability over time can be expressed in the epicarp and mesocarp.

To control pathogens cocoa pod in regard to BCA, research is developing, so is the case that Evans *et al.*, 2003b classified numerous promising candidates for control of *Monilia* in *Theobroma gileri* in western Ecuador, especially *Clonostachys* spp. and *Trichoderma* spp.

The inoculum vehicle (vegetable oil) in this study found to be consistent in its expression, for palm oil, soy and agriculture, which showed the most favorable responses regarding colonization percentage (over 50%) (Figure 3), regardless of the origin of plant material, the form (epiphytic and endophytic) and the time of evaluation.

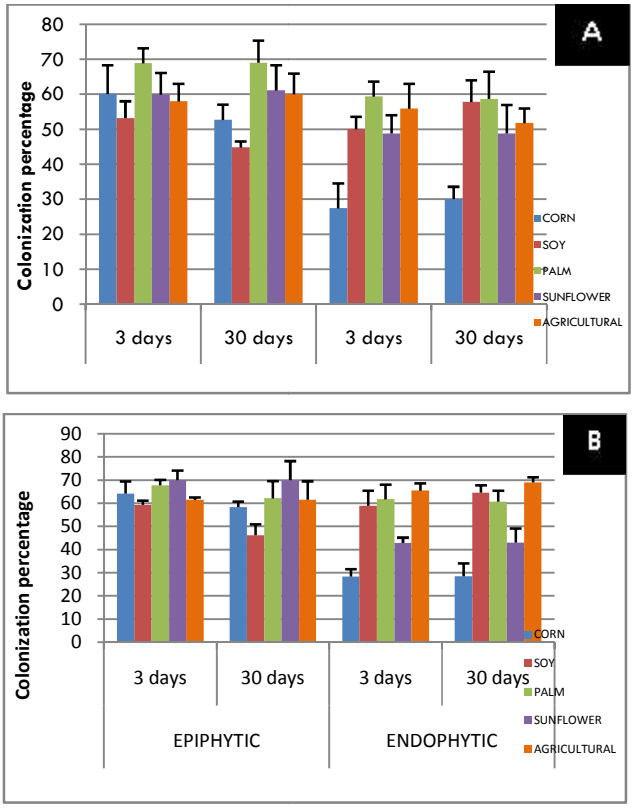


Figure 3 Percentage of Colonization BCA vehicle under the influence of inoculums (vegetable oils) in different materials cacao (Trinitarian and National) in two seasons (A: June B:January).

Formulations in field applications in Costa Rica showed that *T. ovalisporum* Samuels (K-1) could be re-isolated from cocoa pods ten weeks after inoculation and an adverse effect on sporulation of *M. roreri* (Evans *et al.*, 2003a; Holmes *et al.*, 2006). Inoculations addressed are the potential for colonization of tissues cacao tree weapon, as was achieved with the help of a formulation based on palm oil which had higher control values, 59% (Arnold and Herre, 2003). The oil-based formulations clearly improve the adhesion of

organisms to the tissues of the cacao plant such as floral bearings and the ears particular case, the main objective of the study in these trials, where the recoveries are very promising.

There is a particular expression of the BCA inoculum corn oil vehicle which has the smallest response as to the particular colonization endophytic associated intervention.

Influence of Climatic Factors in the Persistence of BCA

The relationship between climatic factors directly influences over time in the persistence of BCA. From the results we can show that the heliophany and precipitation are the most variable factors in their distribution over time(Figure4), which is critical for persistence and colonization of *Trichoderma*.

Deberdt *et al.*(2008), they found the presence of BCA and its distribution in the plot up to two years after treatment, the results showed that *Trichoderma*propagules consistently maintained inpads cacao tree flowers; these authors argue that the use of antagonistic microorganisms is often considered one of the safest and most affordable control strategies.

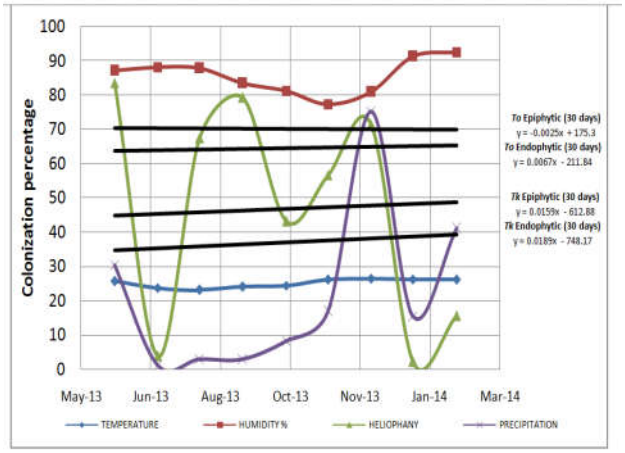


Figure 4 Persistence of BCA influences of climatic factors in different plant materials cocoa (Trinitarian and National).

You need to configure several trials in different agro-ecological conditions, in order to developan effective and lasting method. In parallel, an economic study needs to be conducted in order to show the profit generated by the use of such antimicrobial agents compared to current chemical control.

Biological control of *Moniliophthora*spp. can be classified into two approaches: inundative and classical biological control. Classical biological control was originally developed for the management of insect pests and weeds. For the control of pathogens by inundative type is used to native isolates of the area. The argument for this approach is the adaptation of the agent to local ecological conditions. In order to achieve good control, these agents have to be applied inrelatively large amounts and repeatedly along the selected period. This approach was developed for controlling diseases in Perucocoa pod, which increases were obtained in yield of up to 15% using strains in combination (Krauss and Soberanis, 2001; 2002). In contrast, the classical approach is applicable insituations where a pest has been introduced in tone w regions. In the absence of natural enemies, the new plague eintensifies and becomes a problem, often much more severe than in the country of origin. In such cases, the strategy is to introduce a natural enemy that co-evolved with the pest in the new location to restore the natural balance(Evans, 1999; Bateman *et al.,* 2005; Krauss *et al.,* 2010).

Trichoderma species grow and proliferate better inconditions of high humidity and temperatures between 25 and 30 °C (Klein and Eveleigh, 1998; Sanogo *et al.,* 2002), which was observed in the treatments applied as both evaluation times were optimal with slight variations in the response variable. A clear example of this was demonstrated by Sanogo *et al.*(2002), which determined that *T.stromaticum* requires high humidity (100%) and temperatures below 30 °C for optimal growth. In studies by Holmes *et al.*(2004), cocoa pods were colonized by endophytic *To*wet through simulation chambers. For our evaluations persisting *To*is clearly superior to *Tk* both epiphytic and endophytic level; this is a feature provided native isolates which are highly specific to their ecological niche.

In the ANOVA(Table2); significant differences for treatments were established. When performing statistical significance range sit was found that the highest percentages of colonization in the early days of evaluation, in terms of epiphytic action a rose in the treatment ICS -96 +*Tk*4.09+86% Oil agricultural and endophytic ICS-96 +*Tk*4.09+sunflower oil 88%. By contrast the lowest percentages were recorded for EET - 233 + *To* 70a+18% Oil agricultural and EET– 233 + *To*70a+Sunflower oil with 6%, epiphytic and endophytic respectively action. In the second epoch of the highest expressions evaluation presented them both in epiphytic 100% and endophytic 96% treatment ICS – 96 + *To*70a +soybean oil; the lowest percentages were recorded for EET-233 +*To* 70a+soyoil with 8% epiphytic and EET – 233 + *To*70a+Sunflower oil with 0% endophyte.

Table 2 Significant differences in the percentages of colonization of the treatments in the evaluation of development of an integrated management of diseases ofcacao (*Theobroma cacao* L.) with emphasis on biological control.

TREAT.	Evaluation *in vitro*			
	Colonization percentage			
	June		January	
	Epiphytic	Endophytic	Epiphytic	Endophytic
t1	38 j	28 k	36 i	24 k
t2	46 h	64 f	36 i	52 g
t3	82 b	72 c	68 f	60 f
t4	72 d	88 a	80 d	84 c
t5	86 a	56 h	96 b	92 b
t6	82 b	54 i	92 c	60 f
t7	82 b	76 b	100 a	96 a
t8	82 b	68 e	72 e	60 f
t9	68 e	70 d	68 f	68 e
t10	72 d	72 c	60 g	68 e
t11	5 g	24 l	72 e	16 m
t12	38 j	62 g	44 h	80 d
t13	72 d	76 b	68 f	80 d
t14	80 c	30 j	100 a	20 l
t15	66 f	62 g	68 f	80 d
t16	42 i	14 o	32 j	8 n
t17	18 l	28 k	8 l	32 j
t18	38 j	20 m	44 h	40 h
t19	28 k	6 p	32 j	0 o
t20	18 l	18 n	20 k	36 i

DMS	**	**	**	**

Means with different letters indicate significant differences according to Fisher LSDtest(P<0.05).

In analyzing the treatments peculiarity in the formulation we can say that in general, regardless of the time of application the best answers as to colonization (epiphytic and endophytic) was presented by treatment ICS - 96 + *To*70a+Soybean Oil and lower response in general is to EET -233 + *To*70a+sunflower oil. According to these results we can see that the plant material factor(Trinitarian and National) has high significance in the expression of either the inoculums BCA vehicle.

In the materials evaluated (EET- 103; EET-233, ICS-96 and CCN-51) in applications targeting pods with *Tk* in formulations, it presented less responsive; however not as promising microorganism must discard because their capacity and mico antisporulant hyphal parasitism is one of the best in this species. As there is little or no information on the adaptation of the organism in different cocoa plant materials in different agro ecological zones and this would be one of the initial study demonstrating that materials Nacional cacao type recovery rate is lower compared BCA the Trinity-type factor that must be taken into account for future research.

Acknowledgments

These investigations have been carried out thanks to the contribution of the USDA/ ARS under an agreement for cooperation agreement N° 58-1275-9-198F.

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