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Antagonistic Potential of Native Isolates of *Trichoderma viride* on Corm Rot Pathogen Complex of Saffron (*Crocus sativus*) in Kashmir

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Abstract: Investigation was undertaken to screen the potential native isolate of *Trichoderma viride* for bio suppression of corm rot pathogen complex, as *Trichoderma viride* are the most successful and widely used biocontrol agents. Taking the advantage and constraints of *Trichoderma viride* into consideration, efforts were made to encourage the native isolate against corm rot pathogens. Nine isolates of *Trichoderma viride* namely TK_1, TK_3, TK_4, TK_6, TK_8, TK_9, TK_{10}, TK_{11} and TK_{15} were isolated from soils of different orchard plantations of Kashmir valley on modified *Trichoderma* Specific Medium (TSM). The isolates were studied for their cultural, morphometric characters and antagonistic potential against six newly recorded major fungal pathogens of saffron viz. sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Phytophthora* sp., *Fusarium oxysporum* f. sp. *gladioli*, *F. oxysporum* and *F. solani* individually on Potato Dextrose Agar, the culture morphology of all the isolates was found to be similar. The isolate TK_1, TK_3, TK_4, TK_8, TK_9, TK_{11} and TK_{15}, were found fully overgrown on all corm rot Pathogens of saffron, where as the isolates TK_{13} failed to inhibit the *Phytophthora* sp. Efforts are onto evaluate the performance of promising isolate in field by soil and seed application methods.

Key words: Bio suppression, sterile basidiomycetes fungus, *Rhizoctonia solani*, *Phytophthora* sp., *Fusarium* spp., saffron

INTRODUCTION

During last fifteen years, saffron crop has been affected by severe rotting caused by sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium* f. sp. *gladioli*, *Fusarium oxysporum* and *Fusarium solani* (Mir and Devi, 2004) and reduction in yield has been reported. In 1980 the yield per hectare was 5.66 kg ha^{-1} (Mir, 1992) and now its present productivity is 1.53 kg ha^{-1} (Anonymous, 2009) which is the lowest in the world.

In recent years, attempts were also made to use a consortium of biocontrol agents to get persistent control of plant pathogens (Chaube and Sharma, 2002). Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Antagonistic fungi - 38 - especially *Trichoderma* spp. has been widely used against a number of phytopathogens (Rini and Sulochana, 2006) and parasitized hyphae of other fungi *in vitro* and brought about several morphological changes during destruction (Anitha and Murugesan, 2001). Screening of potential *Trichoderma* strains was done by Bandopadhyay *et al.* (2003) against major root pathogens and it was found that more or less all the strains checked the growth of the pathogen and stimulate plant defensive mechanisms (Hanson and Howell, 2004; Harman *et al.*, 2004; Yadav *et al.*, 2011).

Trichoderma harzianum is one efficient biocontrol that is commercially produced to prevent development of several soil pathogenic fungi (Jegathambigai *et al.*, 2009). Biocontrol is an important approach for plant disease management under changing food habits and commercialization of agriculture (Manczinger *et al.*, 2002).

Therefore, keeping in view medicinal importance and to remove the pesticidal residue of such valuable medicinal crop, the present study was undertaken for screening of several local antagonistic isolates of *T. viride*, obtained from different orchards of Kashmir valley, under *in vitro* conditions against few pathogens sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp., *gladioli*, *Fusarium gladioli*, *Fusarium solani*, *Phytophthora* sp. causing corm rot syndrome of saffron.

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MATERIALS AND METHODS

Collection of pathogen: Four pathogenic isolates namely sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *gladioli*, *Fusarium gladioli*, *Fusarium solani* and *Phytophthora* sp. were isolated, from infected corms from Kashmir valley from saffron growing area. The pathogens were maintained on PDA medium at 4°C.

Isolation of *Trichoderma* spp. (TK_1, TK_3, TK_4, TK_6, TK_8, TK_9, TK_{10}, TK_{11} and TK_{15}) was done from randomly collected soils from different vegetable fields and orchards of Kashmir valley by dilution plate technique using *Trichoderma* specific medium TSM (Elad *et al*., 1981) modified by Saha and Pan (1997).

Antagonistic potential of *Trichoderma viride* isolates on saffron pathogens: The antagonistic properties of fifteen isolates of *Trichoderma viride* were tested on PDA by dual culture plate technique. Paired cultures were observed for a total of 12 days before being discarded. All the ratings were done after contact between pathogen and the antagonist using a Bells scale (Bell *et al*., 1982) which is slightly modified (Class 1-7) as follows.

- S_1 = The pathogen and the antagonism locked at the point of contact
- S_2 = The antagonism starts overgrowth on pathogen.
- S_3 = The pathogens starts overgrowth on mycoparasite
- S_4 = The antagonist overgrew at least 15% of pathogen
- S_5 = The antagonist overgrew of least 30% of pathogen
- S_6 = The antagonist overgrew at least 60% of pathogen
- S_7 = The antagonist completely overgrew the pathogen (100% overgrowth)

RESULTS AND DISCUSSION

Identity of isolates of *Trichoderma* spp.: In general, colony morphology of all the isolates was more or less similar showing sparse to thin colony mycelial mass with whitish border in some cases. Sporulation started after 48h of incubation at 28±1°C for all the isolates (Table 2).

Micrometric measurements of *Trichoderma* viride (Table 1) showed that phialospore length ranged between 2.98-5.52 µm and, breadth ranged from 2.71-4.6 µm and phialides length 9.22-12.56 and breadth 1.3-2.5. These characteristics, particularly the trifid phialophore with short phialides clearly resembled the identical characters of *Trichoderma viride* (Rifai, 1969).

Antagonistic potential of *Trichoderma viride* isolates against corm rot pathogens of saffron.

***Phytophthora* sp.:** The results showed that isolate TK_1, TK_8, TK_4 and TK_{11} were antagonistic to *Phytopthora* by totally overgrowing the pathogen within seven, nine and eleven day respectively. Isolate TK_{10}, TK_{15} and TK_3, TK_6 and TK_9 were antagonistic to Phytopthora overgrowing 75, 45 and 60%, respectively.

***F. oxysporum* f. sp. *gladioli*:** The results showed that isolate TK_1, TK_4, TK_6, TK_8, TK_9 and TK_{11} were antagonistic to *F. oxysporum* f. sp., *gladioli* by totally overgrowing the pathogen within 8 to 12 days. Isolates TK_3 and TK_{10} were overgrowing the pathogen 90 and 45%, respectively.

Sterile Basidiomycetes fungus: The results against Basidiomycetes fungus showed that five isolates TK_1, TK_8, TK_{15} and TK_9 and TK_6, totally overgrowing within nine, eight and 12 day, respectively. The remaining isolate TK_{11}, TK_4, TK_3 and TK_{10}, overgrew 90, 75, 45 and 15%, respectively.

Table 1: Micrometric measurement of phialospores, phialides and chlamydospores of isolate

	Conidia (µm)		Phialide (µm)		Chlamydospore (µm)	
Isolate	L	B	L	B	L	B
TK_1	3.50-4.17	2.71-3.22	10.2-11.9	1.3-2.0	11.04-11.05	11.4-11.0
TK_3	4.21-4.87	3.20-3.62	9.41-9.51	2.0-2.4	8.3-11.4	8.3-11.4
TK_4	4.92-5.52	3.62-4.60	9.25-10.12	1.3-1.9	6.1-7.2	6.1-7.2
TK_6	3.79-4.22	3.10-4.52	9.82-10.45	1.8-2.1	8.5-9.6	8.5-9.6
TK_8	3.41-4.04	2.56-3.21	9.22-10.33	1.4-1.9	7.4-8.9	7.4-8.9
TK_9	3.62-3.97	3.11-3.96	9.99-11.22	1.5-1.8	6.2-7.5	6.2-7.5
Tk10	2.98-3.60	2.89-2.79	10.56-12.22	2.1-2.5	8.7-9.2	8.7-9.2
Tk11	4.27-4.62	3.56-3.88	9.66-9.22	2.2-2.4	10.2-11.5	10.2-11.5
Tk15	3.91-4.55	3.89-4.56	11.22-12.56	1.8-2.5	9.5-10.6	9.5-10.6

Table 2: Colony characters of *Trichoderma viride* isolates

Isolate name	After 3 days dia. (cm)	36 h	After 60 h	After 90 h
Tk_1	3.8	White growth appears inoculum, sparse very thin mycelium hardly seen	Sparse 4 cm mycelium growth, media become yellow around inoculum, after light green sporulation	Light green away from inoculum, inner circle sparse and outer circle with dense growth, encircled dense white fluffy mycelium
Tk_3	3.5	White mycelial growth on the inoculum, very thin mycelium surround the inoculum	Compact white mycelium on inoculum, light green sporulation around the inoculum 2.5 cm after raised cottony growth 8.6 cm	Inoculum covered with snow white mycelium surrounded sparse growth, later thick dirty green slightly fluffy raised 1.5 cm, then dark green
Tk_4	6.3	Sparse whitish thick growth	Compact fluffy light green sporulation on older regions	Around inoculum 2 cm dia. Sparse whitish green after 1.5 cm dia. dark green fluffy raised
Tk_6	5	White growth on the inoculum, encircled sparse mycelium	On inoculum white growth, dense lightly sparse fluffy green sporulation 4.5 cm	On inoculum snow white growth, surround dull green sparse 4 cm media, encircled whitish green raised growth
Tk_8	6	Yellow growth on inoculum, surround spares white growth	Yellow growth on inoculum, around 2 cm sparse light green sporulation	Inoculum covered with yellow growth, surrounded dirty green growth 2 cm, encircled with slightly raised growth
Tk_9	3.9	Thin sparse growth 3.9 cm around the inoculum	Yellow growth appears on inoculum sparse light green sporulation appears white dense growth at periphery	Inoculum covered with green growth, surrounded by dark green band encircled by off white mycelium
Tk_{10}	4	Very thin mycelial growth around the inoculum	3-3.5 cm thin mycelial growth around inoculum encircled with compact dark green sporulation	Around inoculum 5-5.3 cm dia. White mycelial growth. Surrounded by sparse whitish green mycelium 1.5 cm dia. encircled by dark green 0.5 cm dia.
Tk_{11}	7	Thick raised white mycelium	Around the inoculum very light green fluffy mycelium encircled by 1 cm dense green sproulation	Around inoculum 5-5.3 cm dia. White mycelial growth. Surrounded by sparse whitish green mycelium 1.5 cm dia. encircled by dark green 0.5 cm dia.
Tk_{15}	6.3	Around inoculum 3 cm white mycelium	Fluffy mycelium like balls, surrounded dark green sporulation	Fluffy mycelial balls with lightly green sporulation

Table 3: Hyperparasitic potential of *T. viride* wild isolates on fungal pathogens of saffron

	Basidiomycetes fungus		*Rhizoctonia solani*		*Phytophthora* sp.		*F. oxysporum* f. sp. *gladioli*		*F. oxysporum*		*F. solani*	
Isolates	D	R*	D	R	D	R	D	R	D	R	D	R
TK_1	3	$9S_7$**	3	$6S_7$	3	$7S_7$	3	$9S_7$	3	87	3	$10S_7$
TK_3	3	$5S_4+S_5$	3	$8S_7$	3	$7S_4+S_5$	3	$11S_6+S_5$	3	$9S_7$	3	$11S_7$
TK_4	3	$11.S_6+S_4$	3	$7S_7$	3	$9S_7$	3	$10S_7$	3	$7S_7$	3	$9S_7$
TK_6	4	$12S_7$	4	$8S_7$	4	$11S_4+S_5$	4	$5S_1$	4	$5S_1$	4	5S1
TK_8	3	$9S_7$	3	$8S_7$	3	$7S_7$	3	$8S_7$	3	$6S_7$	3	$6S_7$
TK_9	3	$8S_7$	3	$6S_7$	4	$10S_6$	3	$9S_7$	3	$8S_5$	3	$7S_5$
TK_{10}	3	$4S_7$	3	$6S_7$	4	$10S_6+S_4$	3	$11S_5+S_4$	3	$9S_5$	3	$7S_4$
TK_{11}	3	$4S_6+S_5$	4	$9S_7$	3	$10S_7$	4	$10S_6+S_4$	3	$9S_5$	3	$7S_5$
TK_{15}	3	$9S_7$	4	$10S_6+S_4$	4	$10S_6+S_4$	3	$9S_7$	3	$9S_7$	3	$8S_7$

D: Days before contact, R: Rating, **: An average of five individual observation. *: The numerical value represents the days required for attaining S_1 to S_7 stage of modified Bell's scale

Rhizoctonia solani: The results showed that all isolates were antagonistic to *Rhizoctonia solani* by totally overgrowing the pathogen with six to nine day except isolate TK_{15} it overgrew only 75% even after day.

***F. oxysporum*:** The results showed that isolate TK_1, TK_3, TK_4, TK_8 and TK_{15} were antagonistic to *F. oxysporum* by totally overgrowing the pathogen within 6 to 9 days. Isolates TK_9, TK_{10} and TK_{11} did not progress beyond 30% ever after day. The remaining isolate TK_6 totally fails to overgrow the host pathogen even upto 12 days of inoculation inspite of attaining the point of contact of the third day.

***F. solani*:** The result shows that five isolates TK_1, TK_3, TK_4, TK_8 and TK_{15} were highly antagonistic to *Fusarium solani*, totally overgrowing the pathogen within 6 to 11 days. Isolates TK_9 and TK_{11} were overgrew the pathogen 30% whereas TK_{10} 15% and TK_6 failed to overgrew the host pathogen even after 12 days of inoculation, in spite of attaining the point of contact on the 4th day of inoculation.

The overview of the results (Table 3) showed that the isolates TK_1, TK_3, TK_4, TK_8, TK_9, TK_{11} and TK_{15}, were found fully overgrown on all corm rot Pathogens of saffron, where as the isolates TK_{13} failed to inhibit the *Phytophthora* sp. To identify then, isolates of

Trichoderma spp. have been listed in the tables that reached class-I (S_7) stage within 6-11 days of inoculation. However, based on this information the antagonistic *Trichoderma viride* did not allow an early selection of isolates, as the variability in the antagonistic characteristic within the isolate and isolate-pathogen interaction was very high.

The above observations established the fact that Trichoderma isolates existing in their natural conditions in natural ecosystem do differ with respect to their growth and antagonistic potential. Similarly Li *et al.* (2001) studied eighteen isolates of *Trichoderma* spp. of these isolates, TR13 showed greatest antagonists effects against *Rhizoctonia solani.* Several research papers that have appeared in the literature do reveal the fact that various species and isolates of fungal antagonist Trichoderma suppress mycelial growth, reduce root rots, increase plant growth and induce resistance in various crops with which *Sclerotium rolfsii* (Tian *et al*., 2001; Das and Dutta, 2002; Palomar *et al*., 2002), *Rhizoctonia solani* (Li *et al*., 2001; Burgess and Hepworth, 1996; Zapata *et al*., 2001; Ziedan and Mahmoud, 2002; Gaikwad and Nimbalkar, 2003; Yossen *et al*., 2003; Fravel and Lewis, 2004; Hajlaoui *et al*., 2001; Singh *et al*., 2003; Huang and Erickson, 2004; Salehpour *et al*., 2005) are associated. It is clear that the success of bioagents introduces in soil does not guarantee the control the target pathogen(s) because plants, physicochemical and biological factors of soil affect establishment, proliferation and antagonistic activities of the introduced bioagents. It is necessary that, identified antagonist efficiency against foot, root rot and damping off should be investigatited and examined *in vivo* conditions also, the results of such survey would be reported by the authers in near future (Shaigan *et al*., 2008).

It is in this context that to ensure success of introduced bioagents, they should be isolated for the local areas where they exit. Since, they have already faced various processes of evaluation, their application would be feasible and result oriented. We reviewed the literature to find out that have others worked on these aspects. Literature analysis revealed that comparative studies have been done with various species (Kucuk and Kivanen, 2003; Chang *et al*., 2006) studied *Trichoderma* isolates from different soil sampled and grouped them according to their antagonistic potential and chitin utilization.

CONCLUSION

The overgrowth by the antagonist under *in vitro* conditions may be good criteria of selecting an isolate shows good performance under *in vitro* conditions. The trend of the results also indicated that there was not only variability amongst the isolates of *Trichoderma viride* with differential degree of a ntagonism towards a single pathogen but also towards different pathogens.

The results of the study are the pointer to the fact that the antagonists should be isolated from different systems and locations to create a huge genetic pool and tested for their antagonistic potential against variety of the targeted plant pathogens and recommended specifically for different locations and systems. The present study clearly indicates the high potential of biocontrol agent, *Trichoderma viride* isolates for different plant pathogens. Efforts are onto evaluate the performance of promising isolate in field by soil and seed application methods.

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REFERENCES

Anitha, R. and K. Murugesan, 2001. Mechanism of action of *Gliocladium virens* on *Alternaria helianthi*. Indian Phytopathol., 54: 449-452.

Anonymous, 2009. Directorate of Agriculture. Lalmandi, Srinagar, Kashmir.

Bandopadhyay, S., N.D. Sharma and S. Dutta, 2003. Screening of potential *Trichoderma* strains against major root pathogens. Ann. Plant Protec. Sci., 11: 163-163.

Bell, D.K., H.D. Wells and C.R. Markham, 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72: 379-382.

Burgess, D.R. and G. Hepworth, 1996. Biocontrol of sclerotinia stem rot (*Sclerotinia minor*) in sunflower by seed treatment with *Gliocladium virens*. Plant Pathol., 45: 583-592.

Chang, K.F., S.F. Hwang, H. Wang, G. Turnbull and R. Howard, 2006. Etiology and biological control of sclerotinia blight of coneflower using *Trichoderma* species. Plant Pathol. J., 5: 15-19.

Chaube, H.S. and J. Sharma, 2002. Integration and interaction of solarization and fungal and bacterial bioagents on disease incidence and plant growth response of some horticultural crops. Plant Dis. Res., 17: 201-201.

Das, B.C. and P. Dutta, 2002. Management of collar rot of tomato by *Trichoderma* spp. and chemicals. Indian Phytopathol., 55: 235-237.

Elad, Y., I. Chet and Y. Henis, 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica, 9: 59-67.

Fravel, D.R. and J.A. Lewis, 2004. Effect of label and sub label rates of metamsodium in combination with *Trichodema amatum*, *T. harzianum*, *T. virens*, *T. viride* on survival and growth of *Rhizoctonia solani*. Phytoparasitica, 32: 111-118.

Gaikwad, A.P. and C.A. Nimbalkar, 2003. Mangement of collar and root rot (*Rhizoctonia solani*) of bell pepper with bioagent (*Trichoderma* spp.) and fungicides. J. Maharastra Agric. Univ., 28: 270-273.

Hajlaoui, M.R., D. Diop and M. Cherif, 2001. Contribution to biological control of Sclerotinia blight cause by *Sclerotinia sclerotiorum* (Lib.) de Bary. Al Awamia, 104: 85-101.

Hanson, L.E. and C.R. Howell, 2004. Elicitors of plant defence responses from biocontrol strains of *Trichoderma virens*. Phytopathology, 94: 171-176.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito, 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol., 2: 43-56.

Huang, H.C. and R.S. Erickson, 2004. Effect of soil treatment of fungal agents on control of apothecia of *Sclerotium sclerotiorum* in canola and safflower fields. Plant Pathol. Bull., 13: 1-6.

Jegathambigai, V., R.S.W. Wijeratnam and R.L.C. Wijesundera, 2009. Control of *Fusarium oxysporum* wilts disease of *Crossandra infundibuliformis* var. Danica by *Trichoderma viride* and *Trichoderma harzianum*. Asian J. Plant Pathol., 3: 50-60.

Kucuk, C. and M. Kivanc, 2003. Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. Turk. J. Biol., 27: 247-253.

Li, M.Y., G.J. Wang, T.F. Li and K. Liu, 2001. Selection for *Trichoderma* isolates applicable in biocontrol of major fungal diseases of tobacco. J. Southwest Agric. Univ., 23: 10-12.

Manczinger, L., Z. Antal and L. Kredics, 2002. Ecophysiology and breeding of mycoparacitic *Trichoderma strains* (a review). Acta Microbiol. Immunol. Hungarica, 49: 1-14.

Mir, G.H. and L.S. Devi, 2004. Saffron corm rot and their management. Proceedings of the National Symposium on Detection and Management of Plant Diseases using Conventional and Modern Tools and IPS Zonal Meeting (MEZ), Dec. 31, Lucknow, pp: 20-20.

Mir, G.M., 1992. Saffron Agronomy in Kashmir a Study in Habitat, Economy and Society. Gulshan Publishers, Srinagar, Kashmir, India.

Palomar, M.K., Y.C. Mangaoang, V.G. Palermo, G.E. Escuadra and M.B. Posas, 2002. Biocontrol of root crop diseases through microbial antagonism. Proceedings of the 4th Asia-Pacific Biotechnology Congress and 30th Annual Convention of the PSM, May 16-18, 2001, PSMI, College, Laguna, Philippines, pp: 56-62.

Rifai, M.A., 1969. A revision of the genus Trichoderma. Mycological Papers, 116: 56-56.

Rini, C.R. and K.K. Sulochana, 2006. Management of seedling rot of chilli (*Capsicum annuum* L.) using *Trichoderma* spp. and fluorescent pseudomonads (*Pseudomonas fluorescens*). J. Trop. Agric., 44: 79-82.

Saha, D.K. and S. Pan, 1997. Quantitative evaluation of some specific media of *Trichoderma* and *Gliocladium* spp. J. Mycolopathol. Res., 35: 7-13.

Salehpour, M., H.R. Etebarian, A. Roustaei, G. Khodakaramian and H. Aminian, 2005. Biological control of common root rot of wheat (*Bipolaris sorokiniana*) by trichoderma isolates. Plant Pathol. J., 4: 85-90.

Shaigan, S., A. Seraji and S.A.M. Moghaddam, 2008. Identification and investigation on antagonistic effect of *Trichoderma* spp. on tea seedlings white foot and root rot (*Sclerotium rolfsii* Sacc.) *in vitro* condition. Pak. J. Biol. Sci., 11: 2346-2350.

Singh, R., U. Narain and R. Palat, 2003. Evaluation of bioagents against Sclerotinia stem rot of ajowan. Annal. Plant Prot. Sci., 11: 386-386.

Tian, L.S., W.H. Wang, W. L. Shi, S.S. L i, Y.M. Shi, G.W. Zhang and L.P. Zhang, 2001. Studies on mechanisms of antagonism of Trichoderma viride to *Fusarium oxysporum* f. sp. *lycopersici* and its effect of biocontrol. Plant Protect., 27: 47-48.

Yadav, J., J.P. Verma and K.N. Tiwari, 2011. Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian J. Biol. Sci., 4: 291-299.

Yossen, N.A., G.S. Vargas, M. Diaz-P-del and C. Olmos, 2003. Compost and *Trichoderma harzianum* as suppresors of *Rhizoctonia solani* and promoters of let tuce growth. Manejo Integrado Plagasy Agroecol., 68: 19-25.

Zapata, R.L., H.E. Palmucci, V. Blanco-Murray and M.V. Lopez, 2001. Biological trials to control damping-off in eggplant (*Solanum melongena*) with fluorescent *Pseudomonas* and *Trichoderma harzianum*. Rev. Fac. Agron. Univ. Buenos Aires, 21: 207-211.

Ziedan, E.H. and S.Y.M. Mahmoud, 2002. Calcium and sulfur soil treatment to improve biological control with *Trichoderma harzianum* for root rot disease control of bean. Assiut. J. Agric. Sci., 33: 149-160.