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Research Article

Bioefficacy of *Trichoderma* in reducing dead wood formation and enhancing bud sprouting in grapevines

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ABSTRACT: Pasting of the pruning wounds of grapevines with Tricho-XP, a commercial formulation containing *Trichoderma viride* and *Paecilomyces lecanii,* immediately after foundation pruning along with soil application @ 5 g/vine mixed with 245g cattle manure, and spray on the vines @ 1.0 % has shown the potential to reduce dead wood and enhance bud sprouting in Tas-A-Ganesh grapes. Further, five isolates of *Trichoderma* exhibited differences in their antagonism as well as ability to minimize dead wood formation and enhance bud sprouting. *T. harzianum* 5R exhibited maximum *in vitro* antagonism of *Lasiodiplodia theobromae* and was the most effective in minimizing dead wood formation in grapevines. This isolate was able to grow and inhibit radial growth of *L. theobromae* even at higher temperature of 36°C.

KEY WORDS: Pruning wound, *Lasiodiplodia theobromae, Botryodiplodia theobromae, Alternaria alternata, Trichoderma harzianum* 5R

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INTRODUCTION

In the tropical climate of Maharashtra and adjoining states of India the grapevines remain in ever growing state due to lack of cold induced dormancy. To curtail vegetative growth and induce fruitfulness, the vines are pruned twice, once after harvest during the month of April-May and then in October, often termed as foundation and fruit pruning respectively. Since a grapevine bears about 50-60 canes, each pruning creates that many number of pruning wounds, which facilitate the entry of weak facultative parasites.

Further, as a new shoot emerges from the node, the remnant of the internode above this node slowly dies and due to the severity and frequency of pruning, a lot of dead wood accumulates on the arms of the vines. The dead wood gets colonized by facultative parasites, including *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (syn *Botryodiplodia theobromae* Pat.). The problem is more severe in vineyards which are pruned in late April or May due to the prevailing hot weather and in extreme cases it may lead to drying out of the arm. There is no sprouting in portions of the arm where more dead wood is present (Satisha *et al*., 2010) or the arm has dried out, reducing the productivity and the productive life span of grapevines.

Earlier, it was shown that antagonistic fungi can be exploited for the protection of the pruning wounds in grapevines against *Eutypa lata* (Munkvold and Marois, 1993; John *et al.,* 2005; Halleen *et al.*, 2010). The mycoparasites, *Trichoderma harzianum* and *T. viride* are known to inhibit the growth of *L. theobromae* and *A. alternata* also and parasitize it (Batta, 2005, Mortuza and Ilag, 1999; Roco and Pérez, 2001; Sawant and Sawant, 2011). Hence, a commercial formulation of *T. viride* was evaluated for its bio-efficacy in reducing dead wood formation and improving bud sprouting in grapevines in 2005. Subsequently with the aim to identify *Trichoderma* isolates with multiple disease control ablilty in grapes, the antagonistic potential of some promising *Trichoderma* isolates against *L. theobromae,* the dominant colonizer of the dead wood, was tested *in vitro* and their bio-efficacy in preventing dead wood formation and improving bud sprouting was studied in field trials conducted in 2005 and 2011.

MATERIALS AND METHODS

Fungi associated with dead wood and canes

The fungi present in the dead wood and the basal internode of canes (1-year old growth) were isolated from the samples collected from twelve 7-year old vines at the time of foundation pruning in 2005. Twelve random

samples were collected per vine. The dead wood pieces were separated into wood and bark tissues and both were used for isolations. Pieces were surface sterilized by immersing in 0.1 per cent mercuric chloride solution for 45 seconds followed by rinsing thrice in sterile distilled water and then placing in Petri dishes containing 20 ml Potato Dextrose Agar (PDA) (pH 6.5). Plates were incubated at 28±1°C for up to 10 days. The growing edge of fungal growth arising from each piece was sub-cultured on PDA and incubated further for sporulation. The fungi were identified based on morphological features.

Bio-efficacy of a commercial formulation of *Trichoderma*

The trial was conducted after foundation pruning in 2005 in a vineyard trained on bower system in this Centre's vineyard at Manjri on grape variety Tas-A-Ganesh. A commercial formulation of *Trichoderma* i.e. 'Tricho-XP', containing *T. viride* with *P. lecanii* was used for the study. The dead wood present on the vines at the time of foundation pruning was removed before treatment. The formulation was applied as paste to the cut ends, sprayed to cover the arms and to the soil in the drip region immediately after foundation pruning as per details given in Table 2. The trial was laid out in RBD with 4 replications of 12 vines each. Observations were recorded on the central two vines and the outer 10 vines were treated as guard vines. The block was pruned on 31st May 2005 and the maximum temperature (*T*max) from date of pruning to 15 days after pruning was recorded using µMetos automatic weather station. The average *T*max was 36.2 ± 1.9. Data on number of bud sprouts was recorded on 15th day of pruning and on new dead wood at the time of fruit pruning in October.

Table 1. Fungi isolated from dead wood and 1-year old canes collected from Tas-A-Ganesh grapes

Fungus isolated	Isolation frequency (%)		
	From dead wood		From cane
	Wood	Bark	
Lasiodiplodia theobromae	67.5	60.2	34.7
Alternaria alternata	20.0	22.8	0.0
Curvularia sp.	2.5	2.8	0.0
Aspergillus sp.	2.5	0.0	0.7
Phomopsis sp.	0.0	0.0	1.4
Unidentified	0.0	0.0	13.9
No fungal growth	7.5	14.2	49.3
Total	100.0	100.0	100.0

Antagonism of different isolates of *Trichoderma* to *L. theobromae*

Fungal isolates

The *Trichoderma* isolates selected were *T. harzianum* 5R which had known ability to antagonize *L. theobromae* and *A. alternata* (Sawant and Sawant, 2011) and 4 isolates which were obtained from the Culture Collection of NBAIM, Mou, India and had shown ability for control of other fungal diseases of grapes (unpublished data). These isolates were *T. harzianum* (NAIMCC-01741) and *Trichoderma* sp. (NAIMCC-01763) from Soybean from Indore; *T. koningii* (NAIMCC-01938) and *T. pseudokoningii* (NAIMCC-01775) from rhizosphere soil of clove and cowpea respectively from Andaman & Nicobar Islands. The *L. theobromae* isolate was isolated from dead grapevine cane.

***In vitro* parasitism**

The parasitism of *Trichoderma* isolates to *L. theobromae* was studied on Potato Dextrose Agar (PDA) by dual culture method (Dennis and Webster, 1971a). The 90mm PDA dishes were seeded with a disc of *L. theobromae* and *Trichoderma* isolates placed near the edge and on opposite sides and incubated at 28 ± 0.1°C in a BOD incubator (Binder KB 400). Observations on the number of days taken for contact of the two colonies and growth of *Trichoderma* isolate over the *L. theobromae* colony were recorded. Slides were prepared from the zone of interaction, stained with lactophenol cotton blue and observed in bright field at 400x in a Leica DM 2500 compound microscope for lysis of hyphae. The approximate percentage of the mycelium of *L. theobromae* exhibiting lysis of protoplasm was noted for each field. Four observations were taken. The parasitism was also observed at a higher temperature of 36 ± 0.1°C.

Production of toxic volatile and non-volatile metabolites

The production of volatile metabolites was checked by inverting the bottom of a freshly seeded *L. theobromae* plate over a pre-seeded *Trichoderma* 90mm PDA plate (48h growth) and sealing the edges together with a transparent adhesive tape (Dennis and Webster, 1971b). Inoculated *L. theobromae* plate inverted over an uninoculated plate served as control. The production of non-volatile metabolites was checked by growing *Trichoderma* isolates in potato dextrose broth (PDB) at 28 ± 0.1°C in a BOD incubator (Binder KB 400) for

Table 2. Efficacy of a commercial formulation of *Trichoderma* (Tricho-XP) on dead wood formation in Tas-A-Ganesh grapes after foundation pruning

T. No.	Soil application (g/vine)	Pasting of pruning wounds	Spray application	Dead wood (g/vine)	Sprouted buds (no / vine)
T-1	Tricho-XP 5+ FYM 245	Tricho-XP	Tricho-XP 1.0%	9.13	104.25 a
T-2	Tricho-XP 5+ FYM 245	Tricho-XP	Tricho-XP 1.5%	8.94	99.00 ab
T-3	Tricho-XP 10+ FYM 240	Tricho-XP	Tricho-XP 1.0%	5.13	73.00 ab
T-4	Tricho-XP 10+ FYM 240	Tricho-XP	Tricho-XP 1.5%	8.31	57.25 c
T-5	Tricho-XP 5+ FYM 245	Tricho-XP	Tricho-XP 1.0%	11.13	55.13 c
T-6	FYM 250	Bordeaux paste	Bordeaux mixture 1%	7.63	61.50 bc
T7	Control	–	Bordeaux mixture 1%	13.50	33.75 c
	CD (P = 0.05)			NS	39.78

As a general viticultural practice, the arms in all vines, except those in T5, were swabbed with hydrogen cyanamide to induce bud sprouting.

Table 3. Antagonism of different isolates of *Trichoderma* against *Lasiodiplodia theobromae*

Sr. No.	NAIMCC Number	*Trichoderma* isolate	Overgrowth after 10 days (mm)	Per cent degradation of protoplasm	Inhibition of radial growth by	
					volatile metabolites (%)	non-volatile metabolites (%)
1	–	*Trichoderma harzianum* 5R	29.0a	61.75a	38.15c	8.52a
2	1741	*Trichoderma harzianum*	7.0d	25.50d	0.00e	0.00b
3	1763	*Trichoderma* sp.	11.3c	45.00bc	27.41d	6.30ab
4	1938	*Trichoderma koningii*	21.7b	53.25ab	46.67b	0.00b
5	1775	*Trichoderma pseudokoningii*	10.7c	36.00c	55.93a	0.00b
6	–	Control	00.0e	0.00e	0.00e	0.00b
	CD (P = 0.05)		3.17	10.28	7.57	6.89

Table 4. Bioefficacy of different isolates of *Trichoderma*

T. No.	NAIMCC Number	*Trichoderma* isolate	Bud sprouts (May 2011)	Bud sprouts (Oct 2011)	Weight of dead wood (g)	
					April 2011	Oct. 2011
T1	_	*Trichoderma harzianum* 5R	55.0ab	83.5ab	40.63	11.10a
T2	1741	*Trichoderma harzianum*	56.3ab	85.0ab	41.88	19.43c
T3	1763	*Trichoderma* sp.	67.0a	94.5a	40.88	18.63c
T4	1938	*Trichoderma koningii*	49.0b	74.0b	40.00	13.00ab
T5	1775	*Trichoderma pseudokoningii*	52.5ab	77.5ab	44.00	20.80c
T6	–	Control	52.5ab	78.0ab	41.75	17.50bc
	CD (P = 0.05)	17.3	18.6	NS	4.98	

15 days in dark without shaking (stationary phase culture); passing the broth through Grade 42 Whatman cellulose filter paper and subsequent sterilizing by passing through Whatman 13mm 0.2µ nylon syringe filter Puradisc. The sterile cell free culture filtrate was added to sterilized pre-cooled PDA to give a final conc of 10% (v/v) before pouring in petri dishes. The plates were seeded centrally with disc of *L. theobromae.* Plates with unamended medium served as control. In both studies three replications were maintained and the plates were incubated at 28 ±0.1°C as above. Radial growth was measured after 48 h of incubation and percent growth inhibition was calculated.

Bioefficacy of different isolates of *Trichoderma* in field

The trial as per the lay out described earlier was conducted after foundation pruning in 2011 in a vineyard trained on extended 'Y' system in this Centre's vineyard at Manjri on grape variety Tas-A-Ganesh. The isolates were grown on PDB at 28 ± 1°C in natural daylight for 15 days. The broth containing the growth was blended for 15 secs and the count adjusted to 1×10^8 spores per ml by adding appropriate volume of sterile distilled water. Tween 80 was added to derive a concentration of 0.05% (vol/vol). The dead wood present on the vines was removed before treatment at the time of foundation pruning in April and subsequently at the time of fruit pruning in October and fresh weight was recorded. The block was pruned on 18th April 2011 and the *Trichoderma* suspension was applied to the pruning wounds immediately after pruning. The maximum temperature recorded during the experimental period was 36.9 ± 1.5. Data on number of bud sprouts was recorded on 15th day of both foundation and fruit pruning.

Experimental design and statistical analysis

The *in vitro* studies were conducted in a completely randomized block design and the field trials in randomized complete block design. The obtained data was analyzed by one way ANOVA using the statistical package SAS. The percent values were transformed to Arcsine values before analysis.

RESULTS AND DISCUSSION

Fungi associated with dead wood and cane bases

More than sixty percent of the isolations from the dead wood yielded the thermophilic facultative fungi *L. theobromae* (Table 1) indicating that it is the dominant colonizer of the wood tissues corroborating earlier studies (Úrbez-Torres and Gubler, 2011). *Alternaria alternata* was also recorded in the wood tissues with an isolation frequency of about 20%. Frequency of the other two fungi *viz., Curvularia* sp. and *Aspergillus* sp. was only 2 to 3%. About 37% of the isolations from the base of the canes yielded *L. theobromae* (Table 1). One or two isolations yielded *Phomopsis* sp. and *Aspergillus* sp. but *Curvularia* sp. was never recorded. Fourteen per cent of the fungi which did not sporulate could not be identified.

While almost all the dead wood pieces were colonized by one or the other fungi, fifty percent of the canes were colonized mainly by *L. theobromae*. Since these canes are generally used as cuttings for propagation, the finding has significance from plant health management point of view. The association of *B. theobromae* with grapevines in India was also reported earlier (Sawant and Sawant, 2007).

Bioefficacy of a commercial formulation of *Trichoderma*

The dead wood formed in all the treatments though was less than control, are statistically not significant (Table 2), however the trend indicates that regular pasting of the wounds would be beneficial in increasing the productive lifespan of the vines.

The number of bud sprouts was higher in all the treatments as compared to control, which recorded only 33.75 sprouts per vine (Table 2). Treatments T1, T2 & T3 were on par with each and recorded higher sprouts per vine (Table 2). Although bud sprouts in Tricho-XP treatments T4 and T5 were also higher than control, the differences were not significant. Though less dead wood was recorded in Bordeaux pasting (T6) treatment, formation but it did not significantly increase bud sprout, indicating that biological treatments will be a better option to manage the problem.

Antagonism of different isolates of *Trichoderma* against *L. theobromae*

The growing edges of all the five *Trichoderma* isolates made contact with that of *L. theobromae* colony after about 48 hrs of seeding and overgrew it (Table 3). Interestingly, after 2-3 days *L. theobromae* colony started overgrowing on the *T. harzianum* (NAIMCC-1741) colony and suppressed its further growth.

T. harzianum 5R was more aggressive as compared to the other isolates and also caused maximum lysis of the protoplasm. Similarly, all *Trichoderma* isolates, except *T. harzianum* (NAIMCC-1741) produced volatile metabolites inhibitory to the mycelial growth of *L. theobromae.* However, production of inhibitory non-volatile metabolites was minimal and observed only in *T. harzianum* 5R and *Trichoderma* sp. (NAIMCC-01763).

Bioefficacy of different isolates of *Trichoderma* in field

The dead wood at the time April pruning i.e. before imposition of the treatments, varied from 40.00 to 44.00 g per vine, but the differences were not significant (Table 4). However at the time of fruit pruning in October, the differences among treatments were significant. The least weight of dead wood was recorded in those vines where pasting was done with *T. harzianum* 5R (T1). The dead wood in this treatment was on par to the dead wood recorded in vines pasted with *T. koningii* (T4), but significantly less than all other treatments. The number of sprouts was significantly higher in T3 i.e. wounds pasted with *Trichoderma* sp. (NAIMCC-01763) as compared to T4 i.e. wounds pasted with *T. koningii* after both foundation and fruit pruning, though the sprouting in all treatments was on par to control (Table 4).

Earlier Munkvold and Marois (1993) had shown that fungi can be exploited for the biological control of Eutypa dieback of grapevines. Subsequently John *et al.* (2005) and Halleen *et al.* (2010) have also shown that biological control of Eutypa dieback of grapevines is possible using a mixture of strains of the *T. harzianum* product 'Vinevax'. Harvey and Hunt (2006) showed that after application of Vinevax, *T. harzianum* slowly penetrates into the wood, as would be expected from a necrotroph and colonises the dead tissues.

At 36°C, *T. harzianum* 5R and *T. koningii* showed more rate of growth as compared to the other three isolates (data not presented) and these two were also the most effective in reducing dead wood. Interestingly they suppressed the radial growth of *L. theobromae* by about 45% but did not overgrow it.

Results indicate that dressing of wound inflicted during foundation pruning with *Trichoderma* can minimize dead wood and enhance bud sprouting, and can thus help to improve the productivity and productive lifespan of the grapevines in the tropical viticultural areas. *Trichoderma* applications would have advantage over fungicide pasting, as being a saprophyte it would colonize the dead wood and thus its protective effects will be longer lasting as compared to that of a chemical. For best results, the wound dressing should be practiced as a preventive measure from the first year of planting itself.

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