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Screening of Phylloplane and Fructoplane Epiphytes from Grapes for Antagonism against Postharvest Pathogens

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ABSTRACT

Grapes (*Vitis vinifera* L.) is a popular horticultural crop which is vulnerable to a variety of infections during pre and post-harvest conditions. Presently management of these diseases is by the application of synthetic fungicides but non-chemical control methods have gained importance recently in reducing the postharvest decay. Among the epiphytic microflora (29 bacterial and 2 yeast) isolated from phylloplane and fructoplane of the grapes, screened for their antagonistic activity *in vitro* against grapes postharvest pathogens *viz*., *Penicillium citrinum, Alternaria alternata* and *Colletotrichum gloeosporioides*, 15 bacterial and 2 yeast isolates inhibited the growth of all three tested pathogens. These antagonists were found effective and can be utilized for the management of post-harvest diseases of grapes.

Keywords : Antagonism, Biocontrol agents, Epiphytes, Grapes

GRAPES (*Vitis vinifera* L.) is a commercially important tropical fruit crop in the *Vitaceae* family. A considerable amount of grapes production is exposed to post-harvest losses at various stages from production to marketing. The quantum of loss is influenced by several factors like method of harvesting, packing, transportation and attack of pests and diseases. Grapes being a high value commercial crop, any loss could result in significant revenue loss and deprives availability to a large segment of population, causes huge economic loss to the nation (Vilas *et al*., 2011).

As grapes is a perishable commodity, it suffers from both qualitative and quantitative losses before and after fruit harvest. Because of the favourable fruit storage conditions and low resistance mechanisms in plants, fungal infections are able to cause significant damage. *Alternaria* sp., *Aspergillus* sp., *Botrytis* sp., *Colletotrichum* sp., *Pencillium* sp. and *Rhizopus nigricans* are most common post-harvest pathogens. Synthetic fungicides are commonly used in the management of pre and post-harvest fungal diseases, because of their high efficiency. Due to the development of resistance in many postharvest pathogens, several fungicides that are still accessible for use, such as benzimidazole and dicarboximide fungicides are losing their potency (Banoo *et al*., 2020). Furthermore, due to the development of fungicide resistance, public concern about fungicide residues in food, environmental risks and a dearth of fungicide replacements, there is need for alternate measures. Several microbial bio-control agents have been found to manage postharvest deterioration of fruits and concerted efforts has been made in developing alternatives to synthetic fungicides.

Epiphytes are the microorganisms that live naturally on surfaces of fruits (fructoplane) and leaves (phylloplane) and can be employed as antagonists to treat a variety of plant diseases. The feasibility of using mixtures of bacterial and yeast antagonists for the

control of *P. expansum* on apples and suggested several modes of action employed by these microorganisms. Although, microbial antagonists can be applied either before or after harvest, postharvest applications are more effective than pre-harvest applications (Janisiewicz *et al*., 1987).

The current study was conducted with the objectives *viz*., to isolate the most common epiphytes from the surface of grapes leaves and fruits and to evaluate the efficacy of the epiphytes against the most common postharvest pathogens of grapes.

Material and Methods

Isolation of Post-Harvest Pathogens

Grapes fruit samples were collected from different grapes fields in ICAR - IIHR, Bengaluru, Karnataka, India. The diseased and decayed grapes were identified by visual examination and collected in plastic bags. The decayed grapes were initially surface disinfected with one per cent (w/v) sodium hypochlorite solution for one minute and washed in sterile distilled water three times. The decayed portions were plated on petri dishes containing Potato Dextrose Agar medium (PDA) prepared with 200 g of boiled potato, 20 g dextrose, 20 g agar in one litre water and the plates were incubated at 25°C for five days. After that, the resulting fungal colonies were grouped according to their colony morphology, sporulation and representative colony of particular group was selected to obtain the pure culture. Further sub-culturing and maintenance of the isolated fungi were carried out using PDA medium.

Confirmation of Pathogenicity of the Isolated Post-Harvest Pathogens

The pathogenicity of the identified fungal pathogens was determined by inoculating the fungal conidia into healthy grapes as described by Fao *et al*. (2017) with slight modifications. Conidia were harvested from a 10-day-old culture on PDA plates to make the fungal spore suspensions (1×10^5 spores/mL). A 10 µL spore suspension was inoculated into the small wounds made in the grapes berries using needle, whereas berries inoculated with 10 µL of sterile water served as control. The inoculated berries were incubated at 20°C for 14 days in completely closed plastic containers to maintain high humidity. The fruits were checked for disease lesions after the incubation period. The fungus was isolated from the grapes berries infected with disease lesions and found to have identical morphological characteristics to the original isolates. The fungal pathogens that met Koch's postulates were chosen for further research.

Isolation of Phylloplane and Fructoplane Epiphytes

Fruits and leaves were collected during December 2020 to March 2021 from randomly selected grapes vines and mango orchard at Bengaluru, Karnataka. With each sampling, 10 undamaged leaves and fruits of approximately the same size were picked. The leaves and fruits were handled only by the petiole, placed in sterile plastic bags and taken to the laboratory for immediate processing by surface sterilization using water to remove dirt on their surface. Twenty discs, each of 5 mm diameter were cut from every leaf using a sterile 5 mm cork borer and ten grapes berries were separated from the bunch. Leaf discs and grapes berries of respective places were transferred to 250 ml of conical flasks containing 100 mL sterilized distilled water and placed on a rotary shaker at 200 rpm for 30 min to dislodge all epiphytes in the sterile distilled water (Banoo *et al*., 2020).

A serial dilution were made for each sample solution up to 10^{-10} and 0.1 mL aliquots from each of the dilutions were transferred to sterile Petri plates containing nutrient agar (NA) and yeast extract peptone agar (YEPDA) medium (20 g dextrose, 20 g peptone, 10 g yeast extract, 20 g agar and 1 L distilled water) supplemented with 0.05 g L^{-1} cycloheximide (Sigma-Aldrich) and 0.05 gL^{-1} chloramphenicol (Sigma-Aldrich), respectively to have selective isolation of bacteria or fungi and to avoid unwanted colonies. Then the aliquots were spread on the surface of the media using L shaped spreader and the plates were properly labelled and incubated in an inverted position at 26 ± 2°C for 48h. The development of colonies was monitored and different colonies were sub cultured and maintained for further studies.

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Identification of the Organisms

Aerial growth, colony colour, margin and microscopic observations with respect to type of mycelium, spore shape, spore bearing structure and other colony characteristics were analysed in the various fungal colonies obtained. All fungal culture characteristics were compared to the descriptions in the standard manuals.

The bacterial colonies were grown at 26 ± 2°C for 48 hours on specific media NA plates. The colony morphology was studied on plates after streaking a loop full of isolated colony. The effective bacterial isolates were Gram stained observed under microscope at 40X magnification. Cell size, shape and Gram reaction was observed. The bacterial cultures were examined for various morphological, biochemical and physiological characteristics as per the procedure described in Bergey's manual of Determinative Bacteriology.

Identification of yeast isolate was carried on the basis of standard morphological and biochemical tests presented by Barnett *et al.* (2000), Kurtzman and Fell (2006), Rose and Harisson (1987-1993). Other additional test such as addition of 0.05 g L^{-1} chloramphenicol to the YEPDA medium confirms the colony of yeast.

Evaluation of Bacterial and Yeast Epiphytes for their Antagonistic Property

***In vitro* Screening of Bacterial and Yeast Epiphytes for Antagonism**

All bacterial and yeast isolates were screened *in vitro* for antagonism against the most important grapes postharvest pathogens by dual culture method. Five mm mycelial disc of the 10-day old pathogen culture were placed on the periphery about one cm from the edge of the Petri plate (90 mm diameter) whereas, on the opposite direction challenging isolates were streaked using the loop under controlled conditions. Petri plates in which pathogen cultures were not confronted with epiphytes served as control. All the treatments were triplicated and incubated at 24 ± 2^0C for 10 days. After incubation when the growth of the pathogen reached the periphery of the plate in the control, the colony diameter (mm) of the pathogens was measured in each treatment. In case of *Penicillium* sp. due to non-uniform growth, area covered by pathogen on the surface of the plate is calculated. From the measured values, per cent inhibition of the pathogen over control was calculated by using the formula given by Vincent (1947).

$$I = \frac{C - T}{T} \times 100$$

Where;

I = Per cent inhibition

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

Per cent growth inhibition was categorized on a scale given by Korsten (1995) from 0 to 4 *i.e.,* 0 % = 0, 1 to 25 % = 1, 26-50 % = 2, 51-75 % = 3 and 76-100 % = 4. Isolates that reduced pathogen development by producing a demarcation zone or greater growth inhibition were selected for subsequent evaluation of antagonism on grapes.

Experimental Design and Statistical Analysis

The experiments were conducted in a two factorial complete randomized design (CRD) with three replicates using analysis of variance technique. The data was transformed wherever necessary using ICAR - Central Coastal Agricultural Research Institute, WASP 1.0 software at 1 per cent level of probability.

Results and Discussion

Isolation and Identification of Pathogens

Grapes samples collected from the field at ICAR-IIHR, Bengaluru were inoculated on PDA medium. The obtained fungal pathogens were grouped according to morphological features such as colour of the mycelium, texture, shape of the spore and margin of the colony. Pathogens were also molecularly confirmed by ITS (Internal Transcribed Spacer) region of rDNA of fungal isolates which were amplified by PCR (Polymerase Chain Reaction) with universal primer pairs ITS1 and ITS4. Sequence analysis results of the pathogens were aligned with the published full-

TABLE 1

List of phylloplane and fructoplane epiphytes isolated from grapes

Epiphytes	Part of the vine	Variety	Place of collection
IIHR_GIPB01	Phylloplane	Bangalore Blue	IIHR
IIHR_GIPB02	Phylloplane	Bangalore Blue	IIHR
IIHR_GIPB03	Phylloplane	Dilkush	IIHR
IIHR_GIPB04	Phylloplane	Dilkush	IIHR
IIHR_GIFB01	Fructoplane	Bangalore Blue	IIHR
IIHR_GIFB02	Fructoplane	Bangalore Blue	IIHR
IIHR_GIFB03	Fructoplane	Bangalore Blue	IIHR
IIHR_GMPB01	Phylloplane	Bangalore Blue	Matkooru
IIHR_GMPB02	Phylloplane	Bangalore Blue	Matkooru
IIHR_GMIB01	Inflorescence	Dilkush	Matkooru
IIHR_GMIB02	Inflorescence	Dilkush	Matkooru
IIHR_GSPB01	Phylloplane (Old leaf)	Bangalore Blue	Seethakempanahalli
IIHR_GSPB02	Phylloplane (Old leaf)	Bangalore Blue	Seethakempanahalli
IIHR_GSPB03	Phylloplane (Old leaf)	Dilkush	Seethakempanahalli
IIHR_GKPB01	Phylloplane (Young leaf)	Bangalore Blue	Kollarayanahlli
IIHR_GCFB01	Fructoplane	Fantasy	Chikballapura
IIHR_GSFB01	Fructoplane	Dilkush	Shivkote
IIHR_GSTB01	Tendrils	Bangalore Blue	Shivkote
IIHR_GSTB02	Tendrils	Bangalore Blue	Shivkote
IIHR_GSIB01	Inflorescence	Bangalore Blue	Shivkote
IIHR_GAIB01	Inflorescence	Bangalore Blue	Arohalli
IIHR_GAIB02	Inflorescence	Bangalore Blue	Arohalli
IIHR_GAPB01	Phylloplane (Young leaf)	Bangalore Blue	Arohalli
IIHR_GAPB02	Phylloplane (Young leaf)	Bangalore Blue	Arohalli
IIHR_GLIB01	Inflorescence	Sharath	Linganahalli
IIHR_GLIB02	Inflorescence	Sharath	Linganahalli
IIHR_GLFB01	Fructoplane	Krishna	Linganahalli
IIHR_GLFB02	Fructoplane	Krishna	Linganahalli
IIHR_GLFB03	Fructoplane	Sonalika	Linganahalli
IIHR_GIFY01	Fruits	Bangalore Blue	IIHR
IIHR_MIFY01	Fruits	Totapuri	IIHR

B- Bacteria, Y- Yeast

length sequences in the Basic Local Alignment Search Tool (BLAST) databases in National Centre for Biotechnology Information [NCBI]. Three different pathogens were identified *viz*., *Alternaria alternata*, *Colletotrichum gloeosporioides* and *Penicillium citrinum* and the obtained accession numbers were ON009252, ON009253 and ON009254. Al-Najada *et al*. (2019) isolated and identified different post-harvest pathogens *viz*., *Fusarium oxysporum, Aspergillus nige*r and *Penicillium* sp., *Rhizopus*, *Phomopsis, Pestalotiopsis* and *Botryodiplodia* from spoiled grapes fruits.

Isolation and Identification of Epiphytes

After incubation of plates at 26 ± 2°C for 48h, colonies were present only on the agar plates carrying higher sample dilutions (10^{-1} to 10^{-4}). About twenty-nine different bacterial and two yeast isolates of epiphytes were isolated from the phylloplane and fructoplane surface of grapes (One yeast from mango fruit surface) using serial dilution method**.** These isolates were named according to the part of the grapes vine, variety of the grapes and sample collection place of the grapes (Table 1).

Yeast colonies were differentiated from the bacterial colonies upon isolating yeast on the YEPDA medium supplemented with antibacterial antibiotic chloramphenicol (0.05 gL^{-1}) similarly bacterial colonies were identified on NA medium upon supplementing antifungal antibiotic cycloheximide (0.05 gL^{-1}). The results were in confirmation with the findings of Lorenzini and Zapparoli (2020) by isolating 50 epiphytic bacteria from withered grapes and Annu & Suvarna (2015) isolated yeasts from different fruit crops *viz*., Burmese grapes, custard apple, Amla, Jamun and Carambola. Solairaj *et al*. (2020) isolated different isolates of yeast to inhibit the pathogenic fungi causing postharvest decay in table grapes.

Evaluation of Bacterial and Yeast Epiphytes for their Antagonistic Property

***In vitro* Screening of Bacterial and Yeast Epiphytes for Antagonism**

All the bacterial and yeast epiphytes were screened using dual culture method against all the 3 pathogens isolated from symptomatic grapes (*Alternaria alternata*, *Colletotrichum gloeosporioides* and *Penicillium citrinum*). PDA medium was used for this purpose, since it supported the growth of pathogens, yeast and bacteria. A perusal of the data presented in Table 2 revealed that among 31 epiphytes, 18 bacterial isolates and 2 yeast isolates inhibited *P. citrinum* in the range of 76-100 per cent (category 4 on a scale of 0-4) and rest of the 11 bacterial isolates inhibits at the range of 51-75 per cent (category 3 on a scale of 0-4). The maximum (95.46 %) growth inhibition of *P. citrinum* was recorded by IIHR_GSTB02 which was significantly superior over IIHR_GLIB01 (94.73 %), IIHR_GIFB03 (93.02 %) and rest of the isolates whereas, minimum (54.01 %) inhibition percentage was observed in case of IIHR_GAPB02. The yeast isolates *viz*., IIHR_MIFY01 and IIHR_GIFY01 showed 80.78 and 80.39 per cent inhibition respectively.

The maximum growth inhibition (51-75 %) of *A. alternata* was recorded by IIHR_GIPB04 (65.95 %) followed by IIHR_MIFY01 (64.68 %), IIHR_GIFY01 (58.68 %), IIHR_GSIB01 (57.16 %), IIHR_GSPB02 (54.73 %) and IIHR_GAPB02 (54.26 %) which was categorized in group 3 on a scale of 0-4. Other 6 bacterial isolates showed inhibition in the range of 26-50 per cent (category 2 on a scale of 0-4) and 19 bacterial isolates showed inhibition in the range of 1-25 per cent (category 1 on a scale of 0-4). Least inhibition percentage (1.39 %) was observed by IIHR_GKPB01. The data pertaining to the results on effect of phylloplane and fructoplane epiphytes as

Fig 1: Screening of bio-agents against post-harvest pathogens. a) *Penicillium citrinum*, b) *Alternaria alternata*, c) *Colletotrichum gloeosporioides*

TABLE 2

Efficacy of different epiphytes inhibiting the mycelial growth of *Colletotrichum gloeosporioides* in *in vitro* condition

Phylloplane and fructoplane epiphytes of grapes	Per cent inhibition of *Colletotrichum gloeosporioides*			
	Day's After Inoculation			Mean
	3 DAI	6 DAI	9 DAI	
IIHR_GIPB04	94.286 (76.209)	71.667 (57.869)	55.741 (48.321)	73.898 (59.306)
IIHR_GAIB02	90.476 (72.061)	73.81 (59.249)	57.407 (49.285)	73.898 (59.306)
IIHR_GAPB01	80.952 (64.156)	46.19 (42.837)	35.926 (36.845)	54.356 (47.523)
IIHR_GSPB02	85.714 (67.827)	36.19 (37.002)	28.148 (32.059)	50.017 (45.033)
IIHR_GLIB01	74.286 (59.560)	36.667 (37.286)	28.519 (32.295)	46.491 (43.010)
IIHR_GAPB02	66.19 (54.474)	28.333 (32.177)	22.037 (28.012)	38.853 (38.579)
IIHR_GSTB02	67.714 (55.403)	25.571 (30.392)	19.889 (26.499)	37.725 (37.913)
IIHR_GIPB02	60.476 (51.073)	27.857 (31.873)	21.667 (27.755)	36.667 (37.286)
IIHR_GIPB01	60.952 (51.353)	26.19 (30.797)	20.37 (26.843)	35.837 (36.792)
IIHR_GIPB03	60.952 (51.353)	24.524 (29.699)	19.074 (25.909)	34.850 (36.200)
IIHR_GIFB03	56.19 (48.580)	18.095 (25.188)	14.074 (22.045)	29.453 (32.885)
IIHR_GMPB01	51.905 (46.115)	20.238 (26.749)	15.741 (23.387)	29.295 (32.785)
IIHR_GKPB01	58.095 (49.684)	16.19 (23.738)	12.593 (20.796)	28.959 (32.574)
IIHR_GIFY01	13.333 (21.427)	39.5238 (38.973)	31.481 (34.148)	28.113 (32.036)
IIHR_GMIB01	64.286 (53.328)	10.476 (18.894)	8.148 (16.594)	27.637 (31.732)
IIHR_GAIB01	9.524 (17.985)	39.048 (38.693)	30.37 (33.459)	26.314 (30.878)
IIHR_MIFY01	9.524 (17.985)	40.476 (39.530)	27.592 (31.704)	25.864 (30.584)
IIHR_GSIB01	38.571 (38.413)	21.429 (27.589)	16.667 (24.107)	25.556 (30.382)
IIHR_GMIB02	32.667 (34.876)	22.857 (28.575)	17.778 (24.951)	24.434 (29.639)
IIHR_GLFB03	23.81 (29.221)	27.381 (31.568)	21.296 (27.496)	24.162 (29.458)
IIHR_GSTB01	28.857 (32.509)	22.381 (28.249)	17.407 (24.672)	22.882 (28.592)
IIHR_GMPB02	28.095 (32.025)	21.19 (27.422)	16.481 (23.964)	21.922 (27.932)
IIHR_GIFB01	50 (45.023)	6.667 (14.971)	5.185 (13.169)	20.617 (27.018)
IIHR_GSFB01	36.19 (37.002)	11.905 (20.194)	9.259 (17.724)	19.118 (25.941)
IIHR_GSPB03	30.476 (33.525)	14.286 (22.219)	11.111 (19.481)	18.624 (25.580)
IIHR_GCFB01	23.524 (29.028)	15.476 (23.178)	12.037 (20.311)	17.012 (24.372)
IIHR_GLFB01	28.381 (32.207)	11.905 (20.194)	9.259 (17.724)	16.515 (23.990)
IIHR_GIFB02	23.81 (29.221)	9.524 (17.985)	7.407 (15.801)	13.580 (21.635)
IIHR_GLIB02	20.952 (27.255)	5.714 (13.837)	4.444 (12.176)	10.370 (18.795)
IIHR_GLFB02	20.476 (26.918)	2.381 (8.881)	1.852 (7.826)	8.236 (16.686)
IIHR_GSPB01	20 (26.579)	0.952 (5.602)	0.741 (4.941)	7.231 (15.607)
Mean	45.505 (42.443)	25.003 (30.017)	19.345 (26.106)	29.951 (33.197)
		Epiphytes (E)	Days (D)	E x D
SE m±		0.421	1.352	0.177
CD @ P=0.01		0.511	0.159	0.885

Values in parenthesis are arcsine transformed values

TABLE 3

Efficacy of different epiphytes inhibiting the mycelial growth of *Alternaria alternata* in *in vitro* condition

Phylloplane and fructoplane epiphytes of grapes	Per cent inhibition of Alternaria alternata				
	Day's After Inoculation				Mean
	3 DAI	6 DAI	9 DAI	12 DAI	
IIHR_GIPB04	57.083 (49.097)	69.54 (56.531)	70.667 (57.237)	66.519 (54.674)	65.952(54.330)
IIHR_MIFY01	62.708 (52.389)	66.954 (54.938)	68.889 (56.127)	60.185 (50.903)	64.684(53.567)
IIHR_GIFY01	49.479 (44.724)	63.333 (52.760)	60 (50.794)	61.926 (51.926)	58.685(50.027)
IIHR_GSIB01	35.417 (36.540)	65.517 (54.067)	64.533 (53.476)	63.185 (52.672)	57.163(49.143)
IIHR_GSPB02	44.792 (42.032)	40.517 (39.554)	67.333 (55.170)	66.296 (54.538)	54.735(47.741)
IIHR_GAPB02	47.917 (43.829)	52.874 (46.671)	56.356 (48.676)	59.926 (50.751)	54.268(47.473)
IIHR_GAIB02	22.917 (28.616)	42.241 (40.557)	48 (43.876)	56.852 (48.963)	42.503(40.709)
IIHR_GLFB02	29.479 (32.901)	38.793 (38.543)	42.667 (40.804)	45.074 (42.195)	39.003(38.667)
IIHR_GLIB02	15.104 (22.881)	30.46 (33.515)	43.778 (41.447)	46.889 (43.238)	34.058(35.722)
IIHR_GLIB01	36.458 (37.162)	24.138 (29.441)	25.222 (30.162)	29.148 (32.693)	28.742(32.436)
IIHR_GLFB03	2.813 (9.660)	22.126 (28.073)	40.889 (39.771)	41.148 (39.922)	26.744(31.157)
IIHR_GCFB01	16.667 (24.107)	16.954 (24.327)	30.222 (33.367)	33.889 (35.620)	24.433(29.639)
IIHR_GIPB01	12.5 (20.715)	27.586 (31.699)	28.978 (32.585)	23.556 (29.050)	23.155(28.778)
IIHR_GSPB01	4.688 (12.511)	6.897 (15.233)	34.667 (36.089)	41.481 (40.115)	21.933(27.940)
IIHR_GAPB01	6.25 (14.485)	21.092 (27.353)	23.422 (48.137)	28.926 (32.553)	19.923(31.915)
IIHR_GIFB02	1.042 (5.862)	20.69 (27.070)	24.222 (29.498)	25.926 (30.625)	17.970(25.095)
IIHR_GMIB02	8.333 (16.787)	14.943 (22.752)	19.333 (26.098)	26.296 (30.866)	17.226(24.535)
IIHR_GSIB01	1.563 (7.186)	4.368 (12.070)	59.111 (50.275)	0.37 (3.489)	16.353(23.865)
IIHR_GMPB02	1.563 (7.186)	15.172 (22.936)	16.667 (24.107)	31.296 (34.034)	16.175(23.726)
IIHR_GSTB02	9.167 (17.633)	7.989 (16.427)	15.467 (23.171)	24.741 (29.844)	14.341(22.264)
IIHR_GIPB02	3.646 (11.014)	17.529 (24.764)	19.111 (25.936)	16.667 (24.107)	14.238(22.180)
IIHR_GLFB01	1.042 (5.862)	8.621 (17.083)	16 (23.590)	26.704 (31.131)	13.092(21.223)
IIHR_GMPB01	0.521 (4.141)	12.356 (20.590)	13.333 (21.427)	13.519 (21.584)	9.932(18.380)
IIHR_GSTB01	2.188 (8.511)	4.08 (11.659)	7.556 (15.963)	21.778 (27.832)	8.901(17.367)
IIHR_GIFB01	4.167 (11.785)	7.471 (15.871)	9.111 (17.577)	13.407 (21.490)	8.539(16.999)
IIHR_GSFB01	1.875 (7.874)	8.391 (16.847)	9.111 (17.577)	13.333 (21.427)	8.178(16.625)
IIHR_GIPB03	13.75 (21.777)	1.034 (5.839)	0.889 (5.413)	2.963 (9.917)	4.659(12.472)
IIHR_GIFB03	0.208 (2.615)	6.897 (15.233)	10.444 (18.864)	0.741 (4.941)	4.573(12.353)
IIHR_GMIB01	0.729 (4.900)	5.172 (13.152)	5.333 (13.359)	0.741 (4.941)	2.994 (9.969)
IIHR_GSPB03	1.146 (6.149)	4.31 (11.988)	4.444 (12.176)	0.741 (4.941)	2.660 (9.392)
IIHR_GKPB01	1.667 (7.422)	0.575 (4.351)	3.333 (10.525)	0.01 (0.000)	1.394 (6.783)
Mean	16.028 (23.612)	23.504 (29.015)	30.293 (34.904)	30.459 (33.411)	25.071(30.062)
	Epiphytes (E)		Days (D)		E x D
SE m±	0.807		2.248		0.404
CD @ P=0.01	0.735		0.264		1.471

Values in parenthesis are arcsine transformed values

TABLE 4

Efficacy of different epiphytes inhibiting the mycelial growth of *Penicillium citrinum* in *in vitro* condition

Phylloplane and fructoplane epiphytes of grapes	Per cent inhibition of *Penicillium citrinum*		
	Day's After Inoculation		Mean
	5 DAI	10 DAI	
IIHR_GSTB02	95.811 (78.230)	95.118 (77.274)	95.465 (77.743)
IIHR_GLIB01	95.136 (77.298)	94.331 (76.265)	94.734 (76.772)
IIHR_GIFB03	93.564 (75.342)	92.493 (74.136)	93.029 (74.728)
IIHR_GLIB02	91.668 (73.260)	90.289 (71.879)	90.979 (72.558)
IIHR_GIPB03	90.860 (72.440)	89.344 (70.984)	90.102 (71.699)
IIHR_GSPB03	89.461 (71.093)	87.717 (69.519)	88.589 (70.293)
IIHR_GSPB01	88.645 (70.343)	86.772 (68.707)	87.709 (69.512)
IIHR_GSTB01	88.463 (70.180)	86.562 (68.530)	87.513 (69.341)
IIHR_GMIB02	87.655 (69.465)	85.617 (67.747)	86.636 (68.592)
IIHR_GCFB01	87.610 (69.426)	85.564 (67.704)	86.587 (68.551)
IIHR_GAPB01	85.828 (67.920)	83.496 (66.064)	84.662 (66.978)
IIHR_MIFY01	82.249 (65.115)	79.318 (62.982)	80.784 (64.033)
IIHR_GIFY01	81.885 (64.843)	78.898 (62.686)	80.392 (63.749)
IIHR_GAIB01	80.940 (64.147)	77.795 (61.918)	79.368 (63.017)
IIHR_GSFB01	79.494 (63.106)	76.115 (60.774)	77.805 (61.924)
IIHR_GLFB01	74.590 (59.760)	78.350 (62.302)	76.470 (61.014)
IIHR_GLFB03	74.528 (59.719)	78.290 (62.261)	76.409 (60.972)
IIHR_GIPB02	78.147 (62.161)	74.541 (59.728)	76.344 (60.929)
IIHR_GIPB01	78.147 (62.161)	74.541 (59.728)	76.344 (60.929)
IIHR_GMPB02	78.007 (62.064)	74.383 (59.624)	76.195 (60.828)
IIHR_GIFB01	77.823 (61.937)	74.173 (59.486)	75.998 (60.696)
IIHR_GSPB02	77.513 (61.724)	73.806 (59.246)	75.660 (60.469)
IIHR_GMPB01	77.250 (61.544)	73.491 (59.041)	75.371 (60.276)
IIHR_GIPB04	76.795 (61.234)	72.966 (58.702)	74.881 (59.952)
IIHR_GIFB02	75.797 (60.561)	71.811 (57.961)	73.804 (59.245)
IIHR_GSIB01	74.266 (59.547)	70.026 (56.834)	72.146 (58.175)
IIHR_GAIB02	72.828 (58.613)	68.346 (55.791)	70.587 (57.186)
IIHR_GKPB01	72.144 (58.174)	67.559 (55.308)	69.852 (56.725)
IIHR_GMIB01	71.340 (57.662)	66.614 (54.732)	68.977 (56.181)
IIHR_GLFB02	71.112 (57.517)	66.352 (54.572)	68.732 (56.030)
IIHR_GAPB02	57.502 (49.340)	50.525 (45.324)	54.014 (47.326)
Mean	80.873 (64.098)	78.232 (62.221)	79.553 (63.148)
	Epiphytes (E)	Days (D)	E x D
SE m±	0.161	0.635	0.114
CD @ P=0.01	0.295	0.075	0.417

Values in parenthesis are arcsine transformed values

TABLE 5

Growth inhibition category upon screening of epiphytes agents against important postharvest pathogens of grapes

Epiphytes	Growth inhibition category			
	Colletotrichum gloeosporioides	*Alternaria alternata*	*Penicillium citrinum*	Mean growth inhibition category
IIHR_GIPB01	2	1	4	2.333
IIHR_GIPB02	2	1	4	2.333
IIHR_GIPB03	2	1	4	2.333
IIHR_GIPB04	3	3	3	3.000
IIHR_GIFB01	1	1	3	1.667
IIHR_GIFB02	1	1	3	1.667
IIHR_GIFB03	2	1	4	2.333
IIHR_GMPB01	2	1	3	2.000
IIHR_GMPB02	1	1	4	2.000
IIHR_GMIB01	2	1	3	2.000
IIHR_GMIB02	1	1	4	2.000
IIHR_GSPB01	1	1	4	2.000
IIHR_GSPB02	2	3	3	2.667
IIHR_GSPB03	1	1	4	2.000
IIHR_GKPB01	2	1	3	2.000
IIHR_GCFB01	1	1	4	2.000
IIHR_GSFB01	1	1	4	2.000
IIHR_GSTB01	1	1	4	2.000
IIHR_GSTB02	2	1	4	2.333
IIHR_GSIB01	1	1	3	1.667
IIHR_GAIB01	2	3	4	3.000
IIHR_GAIB02	3	2	3	2.667
IIHR_GAPB01	3	2	4	3.000
IIHR_GAPB02	2	3	3	2.667
IIHR_GLIB01	2	2	4	2.667
IIHR_GLIB02	1	2	4	2.333
IIHR_GLFB01	1	1	4	2.000
IIHR_GLFB02	1	2	3	2.000
IIHR_GLFB03	1	2	4	2.333
IIHR_GIFY01	2	3	4	3.000
IIHR_MIFY01	2	3	4	3.000

Values were categorized on a scale from 0 to 4, where 0 = No growth inhibition 1 = 1 to 25 %, 2 = 26 to 50 %, 3 = 51 to 75 % and 4 =76 to 100%

antagonist to postharvest pathogens of grapes at different days interval are detailed in Table 3, 4 & 5.

C. gloeosporioides was inhibited up to 73.98 per cent by two isolates *viz*., IIHR_GIPB04, IIHR_GAIB02 and 54.35 per cent by IIHR_GAPB01. These were placed in category 3 on 0-4 scale of Korsten. Other 14 isolates showed growth inhibition in the range of 26-50 per cent (category 2 on a scale of 0-4) and other 14 isolates showed 1- 25 per cent of growth inhibition (category 1 on a scale of 0-4).

The data further revealed that *in vitro* growth of *P. citrinum*, was reduced by almost all epiphytes but greater inhibition percentage was observed by first 10 isolates (Table 5) whereas, *C. gloeosporioides* and *A. alternata* were inhibited by first 5 microbial isolates (Table 2 and 3).

Of all microbial isolates evaluated, IIHR_GSPB02, IIHR_GLIB01, IIHR_GAIB02, IIHR_GIPB03, IIHR_GAIB01, IIHR_GAPB01, IIHR_GSTB02, IIHR_GLFB03, IIHR_GSPB03, IIHR_GIPB04, IIHR_GCFB01, IIHR_GLIB02, IIHR_GAPB02, IIHR_GSPB01, IIHR_GLFB02, IIHR_GIFY01 and IIHR_MIFY01 were the common epiphytes capable of inhibiting the growth of all the tested pathogens in the category of 4, 3 or 2 and thus proved most effective. Further, effective bacterial and yeast isolates were selected for subsequent molecular identification using ITS (ITS-1 and ITS-4) and 16S (8F and 1492R) region of ribosomal DNA resulting in the identification of different *Bacillus* sp. and *Hanseniospora* sp., respectively. Sequence analysis results of all the effective isolates were aligned with the published full-length sequences in the Basic Local Alignment Search Tool (BLAST) databases in National Centre for Biotechnology Information [NCBI].

There have been very few reports on grape epiphytes and their involvement in the control of grapes postharvest diseases. Similar observations were made by Vargas *et al*. (2012) wherein they isolated, screened *in vitro* and selected 32 different epiphytic yeast for biocontrol of *Botrytis cinerea* on table grapes. Lorenzini and Zapparoli (2020) also isolated different epiphytic bacteria *viz*., *Bacillus*, *Brevibacillus*, *Curtobacterium*, *Micrococcus*, *Pseudomonas* and *Staphylococcus* from withered grapes and screened for their antagonistic effects on grapes-rotting fungi *viz*., *Botrytis cinerea*, *Penicillium expansum* and *Aspergillus uvarum*. *B. subtilis*, *B. paralicheniformis*, *Paenibacillus polymyxa* were also demonstrated as *in vitro* antagonist against *Fusarium oxysporum* causing wilt disease in guava (Maruti and Sriram, 2021). The occurrence of epiphytic antagonistic bacteria and yeast isolates could reduce the contamination of fungal pathogens during grapes harvesting, marketing and could potentially be of interest for fungal biocontrol in the post-harvest processing of fruits and vegetables.

In conclusion, isolation of epiphytes from the phylloplane and fructoplane of grapes revealed the prevalence of different bacteria and yeast with antagonist activity on many postharvest fungal pathogens. The antagonists were shown to be more or less effective against each pathogen in the current study. The results were authenticated as it was revealed that different antagonists may be better adapted to the variable conditions on leaves and fruits. Therefore, different epiphytic antagonists can be applied for the protection against *P. citrinum, A. alternata* and *C. gloeosporioides*. So, *Bacillus* sp. and *Hanseniospora* sp. were proved to be the most efficient bio-agents with its diverse antagonistic mechanism toward phytopathogen fungi, notably due to biofilms, volatile compound synthesis, hydrolytic enzymes, space and nutrient competition and induction of resistance.

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