

1 ***In vitro* evaluation of fungicides against anthracnose of betelvine (*Piper betle* L.)**

2
3 **ABSTRACT**

4 **Background:** Betelvine is important commercial crop and the most profitable among all cultivated
5 crops, which plays a vital role in the overall livelihood security of farm families. Diseases are the
6 major yield constraints of crop plants. One of the most serious fungal diseases of dragon fruit is
7 anthracnose caused by *Colletotrichum* species. Since less information available on anthracnose of
8 betelvine, this study was undertaken.

9 **Methods:** The efficacy of non-systemic, systemic and combination fungicides were tested against
10 *Colletotrichum gloeosporioides* using poisoned food technique (Vincent 1947) under *in vitro*
11 condition. Six non-systemic fungicides Chlorothalonil 75 % WP, Captan 50 % WP, Mancozeb 75 %
12 WP, Copper oxychloride 50 % WP, Propineb 70 % WP and Copper hydroxide 53.8 % at (250 ppm,
13 500 ppm and 1000 ppm), six systemic fungicides Hexaconazole 5 % EC, Propiconazole 25 % EC,
14 Azoxystrobin 25% SC, Tebuconazole 25.9 % EC, Difenaconazole 25 % EC and Picoxystrobin 22.5
15 % SC at (100ppm, 150ppm, 250ppm) and six combi fungicides Propiconazole 13.9 % +
16 Difenoconazole 13.9 % EC, Tebuconazole 50 % + Trifloxystrobin 25 % WG, Fluopyram 200 g/L +
17 Tebuconazole 200 g/L SC, Fluxopyroxad 250 g/l + pyraclostrobin 250g/L, Fluopyram 250 g/L +
18 Trifloxystrobin 250 g/L SC, Azoxystrobin 16.7 % + Tricyclazole 33.3 % SC at (150ppm, 250ppm,
19 500ppm) were evaluated.

20 **Result:** Among six non-systemic fungicides evaluated against *C. gloeosporioides* which was
21 obtained from the isolated sample and results revealed that the Copper hydroxide gave 69.90 %
22 inhibition which was superior over all other fungicides evaluated and least inhibition was recorded
23 with Mancozeb 40.30 %. Difenaconazole, Tebuconazole were the best systemic fungicides found
24 best with inhibition % of 98.28 and 95.17 when evaluated against *C. gloeosporioides*. Out of the six
25 evaluated combination products, propiconazole + difenaconazole exhibited the highest inhibition rate
26 at 99.78%. Following closely, Fluopyram 200g/L + Tebuconazole 200g/L SC and Tebuconazole
27 50% EC + Trifloxystrobin 25% WG displayed inhibition rates of 89.47% and 87.50% respectively.

28 **Key words:** Anthracnose, Betelvine, *Colletotrichum gloeosporioides*, Fungicide

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INTRODUCTION

31 Betelvine (*Piper betle*L.), widely known as “paan” in the Indian sub-continent, has a long
32 ancient history in India and occupies a significant place in the everyday life of the people as it is
33 used in rituals and as medicine to cure many diseases and disorders. Malaysia is the most probable
34 place of origin of the Betelvine(Chatthopadyay and Maity, 1967). It belongs to the family
35 Piperaceae. Betelvine is a perennial, dioecious, shade-loving, aromatic, evergreen root climber with
36 glossy heart-shaped leaves and white catkin (Hiralal Jana, 2016). It is mainly grown in the tropics
37 and subtropical regions, for its leaves are used as a chewing stimulant. In India, betelvine is grown
38 throughout the country and as an important cash crop in southern parts, mainly in Andhra Pradesh,
39 Karnataka, Kerala, and Tamil Nadu. Betelvine is also cultivated in Assam, Bihar, Madhya Pradesh,
40 Maharashtra, Orissa, Tripura, Uttar Pradesh, and West Bengal with an estimated area of 53,539 ha
41 (Ray, 2008). It is the most important cash crop, and that adequately justifies its nomenclature as the
42 "Green gold of India" (Nutankumar *et al.*, 2014).Betelvine leaves and areca nut are used in many
43 occasions like Hindu religious ceremonies, wedding ceremonies, and pujas. Chewing of pan leaf is
44 an ancient habit that has existed for more than 2000 years (Kumar *et al.*, 2014).The essential
45 diseases challenging betel leaf production are foot rot complex (*Phytophthoraspp*, *Sclerotium*
46 *rolfsii*, *Rhizoctonia solani*, *Macrophominaphaseolina*, and *Pythium vexans*), anthracnose
47 (*Colletotrichum spp.*), leaf rot (*Colletotrichum spp.*), Powdery mildew (*Oidium piperis*) and
48 bacterial leaf spot (*Xanthomonas betlicola*).The symptoms of betel vine anthracnose disease on the
49 stem caused by *Colletotrichum spp.* at first appear as tiny, black, circular specks on the green bark
50 of the stem. If conditions are dry, thenthese specks usually do not increase in size and remain as a
51 black stain on the surface of the stem.The leaf spot disease appears only after the rain and affects
52 only betelvine leaves. The disease infection will not spread to the vine. Environmental factors such
53 as temperature, rainfall, relative humidity, and shade in baroja play vital roles in the disease
54 development. The high relative humidity (92 %) was critical for severe leaf spot disease and led to
55 heavy loss in betelvine crops (Dasgupta and Sen, 1999).

56

MATERIAL AND METHODS

57 The poisoned food technique (Nene and Thapliyal 1993), was followed to evaluate the efficacy of
58 non-systemic, systemic fungicides and combi products in inhibiting the mycelial growth of
59 pathogen. The fungus was grown on potato dextrose agar medium for 12 days prior to setting up the

60 experiment. The potato dextrose agar medium was prepared and melted with the use of microwave
61 oven. The fungicidal suspension was added to the melted medium to obtain the required
62 concentrations on commercial formulation basis of the fungicide. 20 ml of poisoned media was
63 poured in each sterilized Petri plates. Control treatment was maintained without addition of
64 fungicide. Mycelial disc of 5 mm was taken from the periphery of 12 days old colony was placed in
65 the center of Petri plates and incubated at $27 \pm 1^\circ\text{C}$ for 12 days and three replications were
66 maintained for each treatment. The diameter of the colony was measured in two directions and
67 average was recorded. Percent inhibition mycelial growth of the fungus was calculated by using the
68 formula given by Vincent (1947).

$$69 \quad I = \frac{C - T}{C} \times 100$$

70

71 Where,

72 I = Percent inhibition

73 C = Radial growth in control

74 T = Radial growth in treatment (fungicide)

75

76 RESULTS AND DISCUSSION

77 The assessment of fungicides through *in vitro* testing proves to be a convenient method for
78 assessing a substantial array of chemicals, gauging their effectiveness in restraining pathogen
79 growth. This approach swiftly furnishes valuable initial insights into the fungicides' potency
80 against the pathogen, offering a concise timeframe for evaluation. These findings then act as a
81 compass for subsequent field trials. In this ongoing study, a total of six contact, six systemic, and
82 six combined fungicide products were examined against *Colletotrichum gloeosporioides*,
83 encompassing three distinct concentrations.

84 Among six contact fungicides evaluated against *C. gloeosporioides* and Copper hydroxide
85 gave 69.90% inhibition which was superior over all other fungicides evaluated. Which was
86 followed by Copperoxychloride (62.96 %), Chlorothalonil (53.87 %), Propineb (47.85
87 %), Captan (40.83 %) and least inhibition was recorded with Mancozeb 40.30 % (Figure 1).

88 The results were similar with work of Parvathy and Girija,(2016). Copper based
89 fungicides are effective because it kills the pathogen by denaturing proteins and enzymes in cells of
90 pathogens when they come in contact.

91 Among six different systemic fungicides evaluated against *C.*
92 *gloeosporioides* Difenoconazole, Tebuconazole were found best with inhibition percentage of
93 98.28 and 95.17. These results were similar to the earlier reports made by Prashanth *et al.* (2008),
94 Ahmed *et al.* (2014), Parvathy and Girija, (2016) that Difenoconazole and Tebuconazole have
95 highest inhibition percentage on the growth of *C. gloeosporioides*. (Figure 2).

96 The effectiveness of the Triazole fungicides may be attributed to their interference with the
97 biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. In many fungi, ergosterol is
98 essential for the structure of cell wall and its absence cause irreparable damage to cell wall leading
99 to death of fungal cell. A similar study was reported for the effectiveness of Triazoles, which inhibit
100 the sterol biosynthesis pathway in fungi (Nene and Thapliyal, 1993).

101 Out of the six evaluated combination products, propiconazole + difenoconazole exhibited
102 the highest inhibition rate at 99.78%. Following closely, Fluopyram 200g/L + Tebuconazole
103 200g/L SC and Tebuconazole 50% EC + Trifloxystrobin 25% WG displayed inhibition rates of
104 89.47% and 87.50% respectively. These findings aligned with the research by Prashanth *et al.*
105 (2008), Parvathy and Girija, (2016), and Pavithra and Benagi, (2017), (Figure 3).

106 The utilization of combination fungicides effectively curbs the development of fungal
107 resistance to systemic fungicides. This is because systemic fungicides disrupt only a single, or
108 occasionally two, functions within the fungal physiology, which can be easily overcome by a
109 singular mutation. On the contrary, non-systemic protectant fungicides impact numerous
110 aspects of fungal physiology, requiring the fungus to undergo multiple changes in order to
111 develop resistance. As a result, the combination of both systemic and non-systemic fungicides
112 yields superior outcomes.

113 CONCLUSION

114 *In-vitro* efficacy of non-systemic, systemic and combi fungicides done to know their
115 efficiency in suppressing the growth of *C. gloeosporioides* revealed the efficacy of copper
116 hydroxide which showed 69.90 per cent inhibition. In case of systemic fungicides maximum
117 per cent inhibition was recorded in Difenoconazole (98.28 %) and lowest per cent inhibition

118 by Azoxystrobin (48.18 %). While among combi products Propiconazole 13.9 % EC +
119 Difenoconazole 13.9 % EC showed 99.78 % followed by Fluopyram 200g/l + Tebuconazole
120 200g/l SC (89.47 %), Tebuconazole 50 % EC + Trifloxystrobin 25 % WG (87.50 %).

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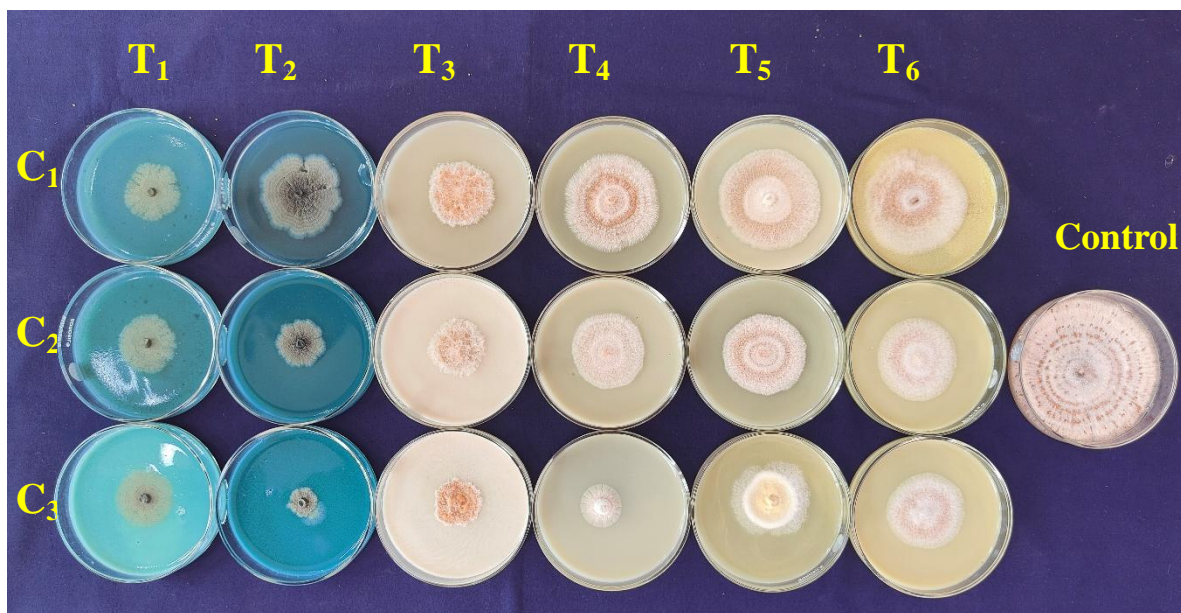
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164 Fig.1: *In-vitro* evaluation of non-systemic fungicides against *Colletotrichum gloeosporioides*

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166 **T1:**Copperoxychloride

167 **T2:**Copper hydroxide

168 **T3:**Propineb

169 **T4:**Chlorothalonil

170 **T5:**Captan

171 **T6:**Mancozeb

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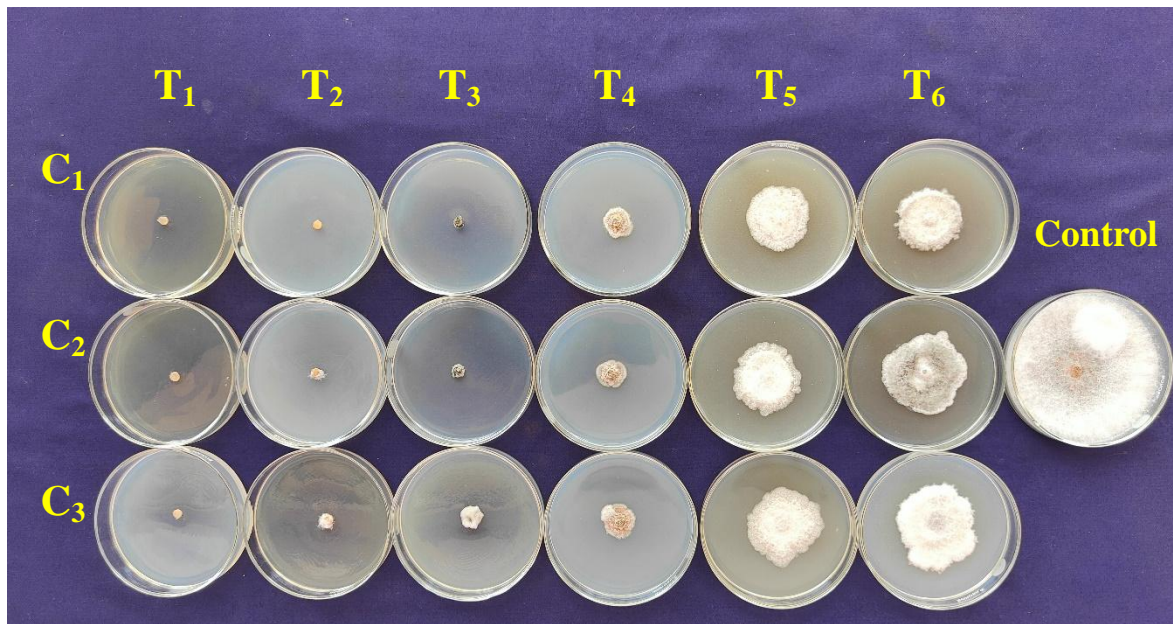
173 **C1:**250 ppm

174 **C2:** 500 ppm

175 **C3:**1000 ppm

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179 Figure 2: *In-vitro* evaluation of systemic fungicides against *Colletotrichum gloeosporioides*

180 **T1:**Difenoconazole

181 **T2:**Tebuconazole

182 **T3:**Propiconazole

183 **T4:**Hexaconazole

184 **T5:**Picoxystrobin

185 **T6:**Azoxystrobin

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187 **C1:**250 ppm

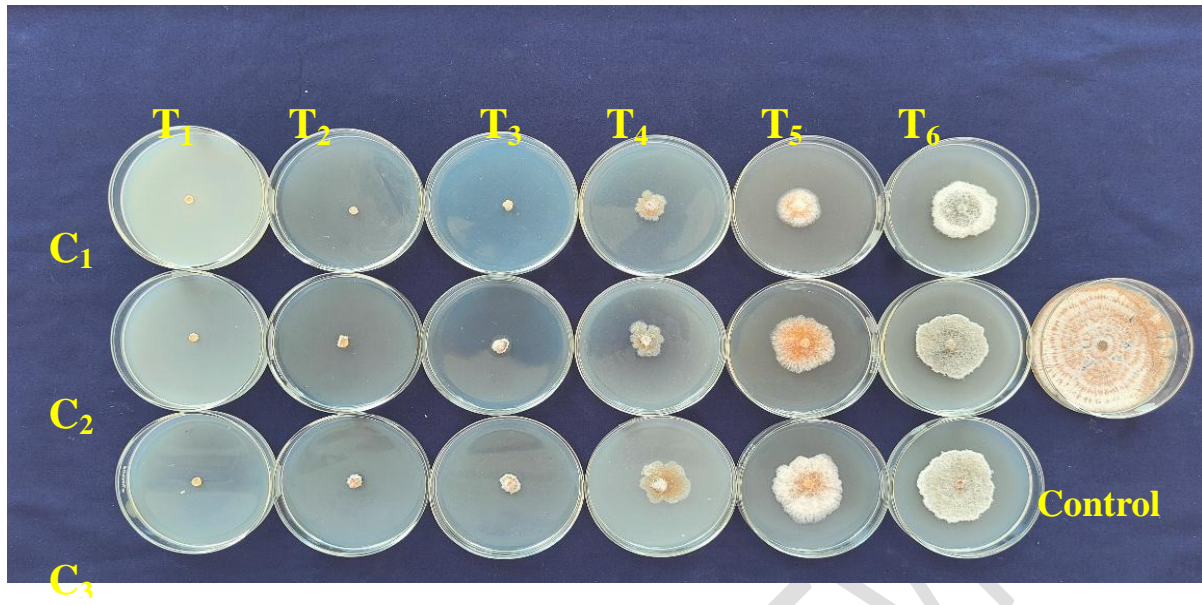
188 **C2:** 150 ppm

189 **C3:**100 ppm

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194 Figure 3: *In-vitro* evaluation of combi products against *Colletotrichum gloeosporioides*

195 **T1:** Propiconazole + Difenoconazole

196 **T2:** Fluopyram + Tebuconazole

197 **T3:** Tebuconazole + Trifloxystrobin

198 **T4:** Fluxapyroxad + Pyraclostrobin

199 **T5:** Azoxystrobin + Tricyclazole

200 **T6:** Fluopyram + Trifloxystrobin

201

202 **C1:** 500 ppm

203 **C2:** 250 ppm

204 **C3:** 150 ppm

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UNDER PEER REVIEW