In vitro evaluation of fungicides against anthracnose of betelvine (_Piper betle_ L.)

ABSTRACT

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

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

Result: Among six non-systemic fungicides evaluated against *C. gloeosporioides*Copper hydroxide gave 69.90 per cent inhibition which was superior over all other fungicides evaluated and least inhibition was recorded with Mancozeb 40.30 per cent. Among six different systemic fungicides evaluated against *C. gloeosporioides*Difenconazole, Tebuconazole were found best with inhibition per centage of 98.28 and 95.17. Out of the six evaluated combination products, propiconazole + difenconazole exhibited the highest inhibition rate at 99.78%. Following closely, Fluopyram 200g/l + Tebuconazole 200g/l SC and Tebuconazole 50% EC + Trifloxystrobin 25% WG displayed

inhibition rates of 89.47% and 87.50% respectively.

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

INTRODUCTION

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

MATERIAL AND METHODS

The poisoned food technique (Nene and Thapliyal 1993) was followed to evaluate the efficacy of non-systemic, systemic fungicides and combi products in inhibiting the mycelial growth of pathogen. The fungus was grown on potato dextrose agar medium for 12 days prior to setting up the experiment. The potato dextrose agar medium was prepared and melted. The fungicidal suspension was added to the melted medium to obtain the required concentrations on commercial formulation basis of the fungicide. Twenty ml of poisoned medium was poured in each sterilized Petri plates. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of 12 days old colony was placed in the center of Petri plates and incubated at 27 ± 1º C for 12 days and three replications were maintained for each treatment. The diameter of the colony was measured in two directions and average was recorded. Per centinhibition mycelial growth of the fungus was calculated by using the formula given by Vincent (1947).

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

Where,

I = Per cent inhibition

C = Radial growth in control

T = Radial growth in treatment (fungicide)

RESULTS AND DISCUSSION

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

Among sixcontact fungicides evaluated against *C. gloeosporioides*Copper hydroxide gave 69.90 per cent inhibition which was superior over all other fungicides evaluated. Whichwas followed by Copperoxychloride (62.96 %), Chlorothalonil (53.87 %), Propineb (47.85 %),Captan(40.83 %) and least inhibition was recorded with Mancozeb40.30 per cent. (Figure 1)

The results were in similarity with work of Parvathy and Girija(2016). Copper based fungicidesare effective because it kills the pathogen by denaturing proteins and enzymes in cells of pathogens when they come in contact.

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

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

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

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

CONCLUSION

In-vitro efficacy of non-systemic, systemic and combi fungicides done to know their efficiency in suppressing the growth of *C. gloeosporioides*revealed the efficacy of copper hydroxide which showed 69.90 per cent inhibition. In case of systemic fungicides maximum per cent inhibition was recorded in Difenoconazole (98.28 %) and lowest per cent inhibition by Azoxystrobin (48.18 %). Whileamongcombiproducts Propiconazole 13.9 % EC + Difenoconazole 13.9 % EC showed 99.78 per cent followed by Fluopyram 200g/l + Tebuconazole 200g/l SC (89.47 %), Tebuconazole 50 % EC + Trifloxystrobin 25 % WG (87.50 %).

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Fig. 1: Symptoms of anthracnose of dragon fruit

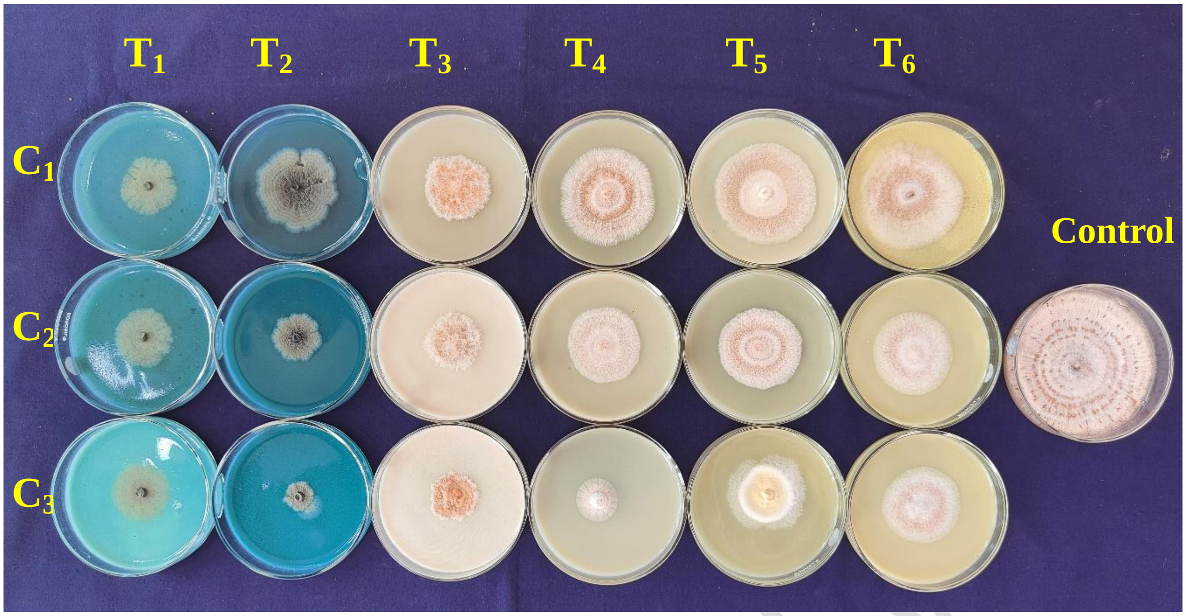

Fig.1: *In-vitro* evaluation of non-systemic fungicides against *Colletotrichum gloeosporioides*

T1: Copperoxychloride

T2: Copper hydroxide

T3: Propineb

T4: Chlorothalonil

T5: Captan

T6: Mancozeb

C1:250 ppm

C2: 500 ppm

C3:1000 ppm

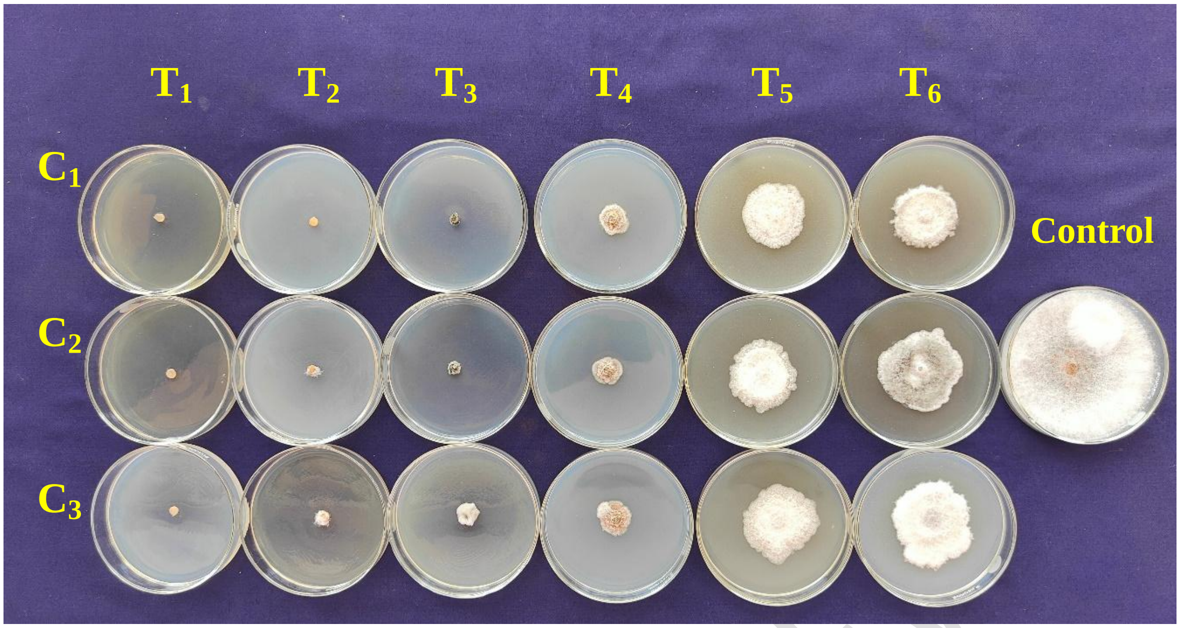

Figure 2: *In-vitro* evaluation of systemic fungicides against *Colletotrichum gloeosporioides*

T1: Difenoconazole

T2: Tebuconazole

T3: Propiconazole

T4: Hexaconazole

T5: Picoxystrobin

T6: Azoxystrobin

C1:250 ppm

C2: 150 ppm

C3:100 ppm

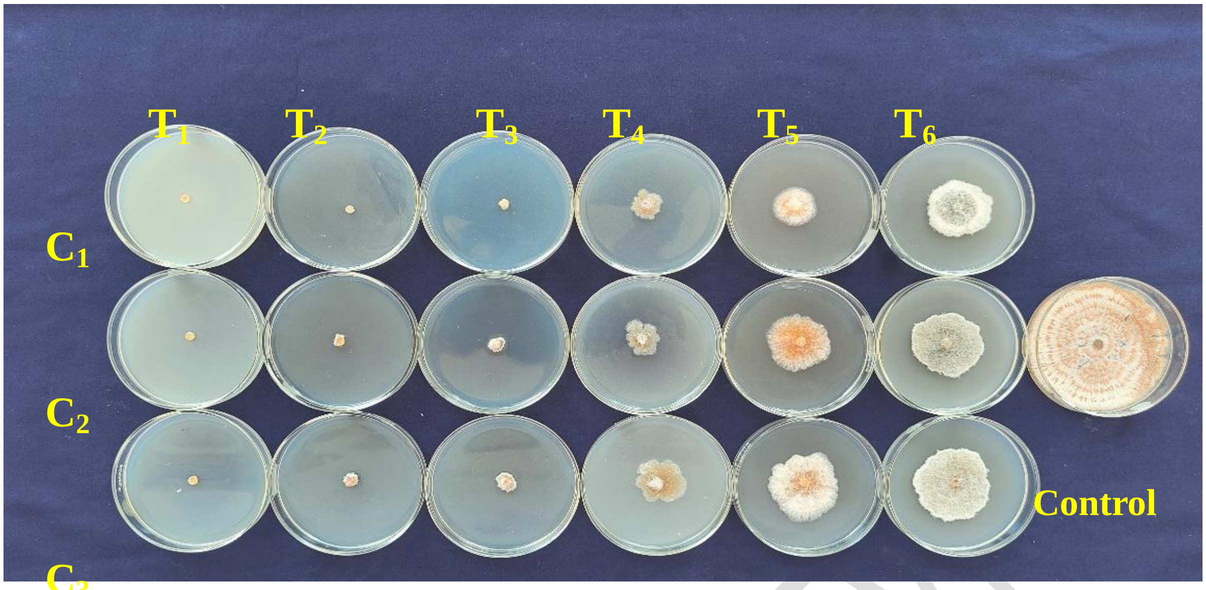

Figure 3: *In-vitro* evaluation of combi products against *Colletotrichum gloeosporioides*

T1: Propiconazole + Difenoconazole

T2: Fluopyram + Tebuconazole

T3: Tebuconazole + Trifloxystrobin

T4: Fluxapyroxad + Pyraclostrobin

T5: Azoxystrobin + Tricyclazole

T6: Fluopyram + Trifloxystrobin

C1: 500 ppm

C2: 250 ppm

C3: 150 ppm