



# **Potential of Bioagents and Botanicals on Vegetative Growth Parameter of Betelvine (*Piper betle*) against Leaf Spot (*Colletotrichum capsici* Syd.)**

**Anoop Kumar <sup>a\*</sup>, Sunil Zacharia <sup>a</sup>, Mukul Kumar <sup>b</sup>,  
Siddharth Singh <sup>c</sup> and Saurabh Kumar <sup>b</sup>**

<sup>a</sup> Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj- 211007 (U.P.), India.

<sup>b</sup> Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur-208002 (U. P), India.

<sup>c</sup> Department of Plant Pathology, Bidhan Chandra Krishi Vishwa Vidyalaya, Mohanpur, Nadia, West Bengal-(741252), India.

## **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## **ABSTRACT**

The betelvine (*Piper betle* L.) is a perennial climbing evergreen shrub belonging to the Piperaceae, also known as the pepper family. As leaves has economic part of betelvine crop and consumed freshly harvested leaves. The present study were carried out at farmer's field in collaboration with Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences Prayagraj to find out the ecological, environmentally and biological safe treatment to manage the disease as well increases the plant height and leaves length. Among all the

\*Corresponding author: E-mail: anoopyadav5190@gmail.com;

treatment *Trichoderma harzianum* found to increase maximum plant height at different days after planting. While, *Trichoderma viride* increases the maximum leaves length and breadth during both the year.

**Keywords:** *Trichoderma harzianum*; *Bacillus subtilis*; anthracnose; *Colletotrichum capsici*.

## 1. INTRODUCTION

The betelvine (*Piper betle* L.) is a perennial climbing evergreen shrub belonging to the Piperaceae, also known as the pepper family; it is cultivated for its leaves. The heart-shaped, deep-green leaves of the betelvine are popularly known as paan in India. Nutritional composition of fresh betel leaf is water (85-90%), Protein (3-3.5%), Fat (0.4-1.0%), Minerals (2.3-3.3%), Fibre (2.3%), Chlorophyll (0.01-0.25%), Carbohydrate (0.5-6.10%), Nicotinic acid (0.63-0.89 mg/100g), Vitamin C (0.005-0.01%), Vitamin A (1.9-2.9 mg/100g), Thiamine (10-70 µg/100g), Riboflavin (1.9-30 µg/100g), Tannin (0.1-1.3%), Nitrogen (2.0-7.0%), Phosphorus (0.05-0.6%), Potassium (1.1-4.6%), Calcium (0.2-0.5%), Iron (0.005-0.007%), Iodine (3.4 µg/100g), Essential Oil (0.08 - 0.2%), Energy (44 kcal/100 g) and medicine for liver, brain and heart diseases. Betelvine leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, constipation, headache, itches, hysteria, mastitis, mastoiditis, ringworm, leucorrhoea, swelling of gum, rheumatism, abrasion, injuries and cuts, etc., as folk medicine while the root is known for its female contraceptive effects Chopra et al. [1]. Chewing of betelvine leaves increased alertness, produces a sense of well-being, sweating, salivation, a hot sensation, and energestic feelings with exhilaration. It also increases the capacity to physical exercise and mental functions more efficacy for a longer duration, but it may produce a kind of psycho-active effect causing a condition of mild addiction leading to habitual and withdrawal symptoms [2,3]. The most probable place of origin of the betelvine is Malaysia Chattopadhyay and Maity [4]. The annual turnover of betelvine in India is estimated at Rs. 10,000 million. Betelvine cultivation is done in major districts of Uttar Pradesh, including Mahoba, Lucknow, Unnao, Raibarely, Sitapur, Pratapgarh, Allahabad, Ballia, Azamgarh, Kanpur Nagar, Banda, Mirzapur, and Sonbhadra. In Bihar, the most common varieties are "Bangla," "Calcuttia," "Magahi," "Kapoori," and "Semehi." This is a high-risk crop both during production and marketing because betelvine leaves are highly perishable and susceptible to

changes in weather conditions, because of which many diseases occur, viz., anthracnose caused by *Colletotrichum capsici* (Syd.) Butler and Bisby, and bacterial leaf spot caused by *Xanthomonas campestris* pv. *betlicola* (Patel, Kulkarni and Dhande) Dye, Phytophthora leaf and foot rot caused by *P. palmivora*, and basal rot caused by *Sclerotium rolfsii* are the main yield-limiting factors of betelvine cultivation all over India. Leaf spot caused by *Colletotrichum capsici* is one of the major crop diseases Bhale et al. [5] that can kill plants and produce up to 25-90% of the crop in different parts of India [6,7]. This disease of betelvine was first identified by and it is also known as anthracnose of 'Pan' (vernacularly called as Jhalma').

## 2. MATERIALS AND METHODS

Experiments were conducted in on farmers field Village – Bhandia, Teh- Sidholi, District- Sitapur, (U.P.), in the year 2016-2017 and 2017-2018 in randomised block design (RBD). The Research field (*in situ*) is situated on the north side of Bhandia. The site selected was uniform, farmable with sandy loam soil having good drainage. The farmer's field selected for experiment is situated at 27.18° North latitude and 80.56° East longitudes and at an altitude of 138 meter above sea level. Experimental plot has sub-tropical and semi-arid climate with the monsoon commencing from July and withdrawing by the end of September. The temperature goes up to 22-40°C during summers and goes down to 10-23°C in winter. Stem cuttings having 3–5 nodes were used for propagation and these were planted in such a manner that 2–3 nodes were buried in the soil. Cuttings of the apical and middle portions of the vine were used for planting [8]. After four months of planting, more soil (Gasti) and bamboo stick (wash) was added. It was done again at two months of intervals. Plant height was measured by the help of inch-tape and the same time length and breadth of leaves were measured by the help of scale and after measuring leaves creeper were bend downward and next upper portion were measured for plant height. Leaves were tagged of which parameter were observed

and per cent increase in length of leaves and breadth were observed.

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Different Bio-agents and Botanicals on Plant Height (cm) of Betelvine at 60 DAP

Potential of different bio-agent and botanicals on plant height were observed at different days after planting viz., 60, 90, 120, 150, 180, 210, 240, 270, 300 and 330 DAP during 2016-17 and 2017-18 (Tables 1-10). The data pertaining in table mentioned below indicated that response of bio-agent and botanical were superior in terms of plant height and there were significant increase in plant height due to application of different bio-agent and botanicals as compared to control plot and check plot with copper oxychloride. The maximum plant height (55.50 cm during 2016-17 and 78.40 cm during 2017-18 with mean value 66.95 cm at 60 DAP was observed in T<sub>2</sub> treatment which was closely followed by T<sub>3</sub> treatment exhibited 53.00 cm and 78.05 cm with mean value 65.53 cm at 60 days after planting. Rate of increase in plant height were also determined at 90 DAP and it was observed that maximum plant height were also recorded in

treatment with *Trichoderma harzianum* (76.75 cm) followed by *Trichoderma viride* (73.65 cm) (Table 1). Among all the treatment the minimum plant height was observed in case of treatment with tobacco leaf extract (70.25 cm). Similarly, similar trend was followed during second year of experiment. As number of leaves depends upon the length of the stem, plant height was further observed regularly at interval of 30 days upto 330 DAP and it was found at 330 DAP that maximum increase in plant height was found in treating the plant with *Trichoderma harzianum* which was statistically at par with *Trichoderma viride* (T<sub>3</sub>). As the length of stem increases, number of leaves increases thereby economic importance of crop as well as economic benefit of farmer will increases. Calvo et al. [9] they were reported that *B. subtilis* bacteria exhibit significant inhibitory effects on the growth of pathogen, *R. solani* by decrease disease incident (%) and promoted vegetative growth of plant and The *Trichoderma* spp. secrete secondary metabolites have proven its role in suppressing the growth of pathogenic microorganisms and stimulating the plant growth [10-13]. Similar result D'Souza et al. [14] reported significant reduction of disease with the use of an isolate of *Trichoderma harzianum*.

**Table 1. Effect of different treatments on plant height (cm) at 60 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment No.	Treatment Details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	35.40	48.35	41.88
T <sub>1</sub>	Copper oxychloride	50.30	66.85	58.58
T <sub>2</sub>	<i>T. harzianum</i>	55.50	78.40b	66.95a
T <sub>3</sub>	<i>T. viride</i>	53.00	78.05b	65.53a
T <sub>4</sub>	<i>Bacillus subtilis</i>	47.30	62.45a	54.88
T <sub>5</sub>	Neem oil	43.85	60.95a	52.40
T <sub>6</sub>	Tobacco leaf extract	42.15	52.35	47.25
S. Ed. (±)		0.662	0.734	0.698
C. D. (P = 0.05)		1.404	1.556	1.479

**Table 2. Effect of different treatments on plant height (cm) at 90 DAP during 2016-2017, 2017-2018 and pooled analysis of betelvine**

Treatment No.	Treatment Details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	49.60	55.75	52.68
T <sub>1</sub>	Copper oxychloride	64.40	81.45	72.93
T <sub>2</sub>	<i>T. harzianum</i>	76.75	96.85	86.80
T <sub>3</sub>	<i>T. viride</i>	73.65	91.25	82.45
T <sub>4</sub>	<i>Bacillus subtilis</i>	60.75	78.65a	69.70
T <sub>5</sub>	Neem oil	58.25	76.45a	67.35
T <sub>6</sub>	Tobacco leaf extract	54.80	69.40	62.10
S. Ed. (±)		1.056	1.113	1.085
C. D. (P = 0.05)		2.240	2.359	2.299

**Table 3. Effect of different treatments on plant height (cm) at 120 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment No.	Treatment Details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	63.55	58.45	61.00
T <sub>1</sub>	Copper oxychloride	77.65a	90.95b	84.30a
T <sub>2</sub>	<i>T. harzianum</i>	91.90b	115.20	103.55b
T <sub>3</sub>	<i>T. viride</i>	91.10b	112.25	101.68b
T <sub>4</sub>	<i>Bacillus subtilis</i>	75.25a	79.30a	77.28
T <sub>5</sub>	Neem oil	74.50a	89.10b	81.80a
T <sub>6</sub>	Tobacco leaf extract	70.25	78.30a	74.28
S. Ed. (±)		1.197	1.282	1.240
C. D. (P = 0.05)		2.538	2.717	2.628

**Table 4. Effect of different treatments on plant height (cm) at 150 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	71.35	91.05	81.20
T <sub>1</sub>	Copper oxychloride	90.10b	125.90	108.00
T <sub>2</sub>	<i>T. harzianum</i>	110.45c	151.05a	130.75b
T <sub>3</sub>	<i>T. viride</i>	108.40c	148.75a	128.58b
T <sub>4</sub>	<i>Bacillus subtilis</i>	88.70ab	119.05	103.88a
T <sub>5</sub>	Neem oil	86.45a	115.40	100.93a
T <sub>6</sub>	Tobacco leaf extract	81.55	98.00	89.78
S. Ed. (±)		1.479	1.620	1.549
C. D. (P = 0.05)		3.136	3.434	3.285

**Table 5. Effect of different treatments on plant height (cm) at 180 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	91.45	117.90	104.68
T <sub>1</sub>	Copper oxychloride	120.25	170.15	145.20a
T <sub>2</sub>	<i>T. harzianum</i>	142.90b	159.35a	151.13
T <sub>3</sub>	<i>T. viride</i>	141.60b	153.00	147.30a
T <sub>4</sub>	<i>Bacillus subtilis</i>	114.00a	166.30	140.15
T <sub>5</sub>	Neem oil	109.60a	161.05a	135.33
T <sub>6</sub>	Tobacco leaf extract	101.40	144.20	122.80
S. Ed. (±)		1.690	1.346	1.435
C. D. (P = 0.05)		3.583	2.853	3.043

**Table 6. Effect of different treatments on plant height (cm) at 210 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	121.90	149.15	135.53
T <sub>1</sub>	Copper oxychloride	165.40	229.85	197.63
T <sub>2</sub>	<i>T. harzianum</i>	188.00a	268.25	228.13
T <sub>3</sub>	<i>T. viride</i>	186.30a	260.10	223.20
T <sub>4</sub>	<i>Bacillus subtilis</i>	156.65	220.10	188.38
T <sub>5</sub>	Neem oil	151.25	207.60	179.43
T <sub>6</sub>	Tobacco leaf extract	145.35	191.25	168.30
S. Ed. (±)		1.972	2.127	2.049
C. D. (P = 0.05)		4.181	4.509	4.345

**Table 7. Effect of different treatments on plant height (cm) at 240 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	155.25	181.25	168.25
T <sub>1</sub>	Copper oxychloride	224.30	259.10a	241.70a
T <sub>2</sub>	<i>T. harzianum</i>	257.70	280.20b	268.95
T <sub>3</sub>	<i>T. viride</i>	250.65	276.45b	263.55
T <sub>4</sub>	<i>Bacillus subtilis</i>	216.00	260.55a	238.28a
T <sub>5</sub>	Neem oil	205.30	242.95	224.13
T <sub>6</sub>	Tobacco leaf extract	196.10	227.65	211.88
S. Ed. (±)		2.251	2.296	2.273
C. D. (P = 0.05)		4.772	4.868	4.820

**Table 8. Effect of different treatments on plant height (cm) at 270 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	188.05	191.70	189.88
T <sub>1</sub>	Copper oxychloride	264.65	299.30	281.98
T <sub>2</sub>	<i>T. harzianum</i>	272.60a	315.35	293.98
T <sub>3</sub>	<i>T. viride</i>	268.05a	308.60	288.33
T <sub>4</sub>	<i>Bacillus subtilis</i>	251.95	285.30	268.63
T <sub>5</sub>	Neem oil	246.35	261.20	253.78
T <sub>6</sub>	Tobacco leaf extract	234.76	240.55	237.65
S. Ed. (±)		2.381	2.521	2.451
C. D. (P = 0.05)		5.047	5.345	5.196

**Table 9. Effect of different treatments on plant height (cm) at 300 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	198.80	200.75	199.78
T <sub>1</sub>	Copper oxychloride	295.20	317.9	306.55
T <sub>2</sub>	<i>T. harzianum</i>	310.25	340.15	325.20
T <sub>3</sub>	<i>T. viride</i>	303.6	331.30	317.45
T <sub>4</sub>	<i>Bacillus subtilis</i>	271.65	301.70	286.68
T <sub>5</sub>	Neem oil	260.85	276.60	268.73
T <sub>6</sub>	Tobacco leaf extract	249.00	250.45	249.73
S. Ed. (±)		2.578	2.747	2.662
C. D. (P = 0.05)		5.465	5.823	5.644

**Table 10. Effect of different treatments on plant height (cm) at 330 DAP during 2016- 2017, 2017-2018 and pooled analysis of betelvine**

Treatment notation	Treatment details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T <sub>0</sub>	Control (Inoculated)	209.85	200.66	205.206
T <sub>1</sub>	Copper oxychloride	315.30a	317.86	316.58
T <sub>2</sub>	<i>T. harzianum</i>	324.90b	339.86	332.38
T <sub>3</sub>	<i>T. viride</i>	320.45ab	331.40	325.93
T <sub>4</sub>	<i>Bacillus subtilis</i>	285.10	301.60	293.35
T <sub>5</sub>	Neem oil	271.40	276.53	273.97
T <sub>6</sub>	Tobacco leaf extract	260.20	250.66	255.430
S. Ed. (±)		2.803	2.817	2.810
C. D. (P = 0.05)		5.943	5.972	5.957

### 3.2 Leaf Length (cm) of Betelvine as Affected by Different Treatments During 2016-2017, 2017-2018

The response of bio-agents and botanicals to the length of leaves was found to increase under field conditions. The maximum increase in length of leaves was increased in treating the crop with copper oxy chloride. The highest length (14.88 cm) during 2016-17 and 15.46 cm) during 2017-18 with mean value 15.17 cm was found in treating the seedling with copper oxy chloride. The data pertaining in the table indicates that there was significant increase in leaves length in all the treatment as compared to control. However, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> are statistically at par with each other. In Pooled data analysis fascinate that highest length (15.83 cm) of leaves was observed in treating the plant with *Trichoderma harzianum*.

### 3.3 Leaf Breadth (cm) of Betelvine as Affected by Different Treatments During May 2016-2017, 2017-2018

The bio-agents and botanicals were used as soil treatment and foliar spray in the field condition. All the treatments are significant over control in

the first year, second year, and pooled data. The breadth of leaves were measured by using scale and it was found that maximum breadth of leaves was observed in case of T<sub>1</sub> treatment *i.e.* treating the crop with copper oxy chloride. Among the treatment with bio-agents and botanicals maximum breadth size during 2016-17 was observed in treating the seedlings with *Bacillus subtilis* (14.47 cm) which was completely followed by T<sub>2</sub> treatment *i.e.* treating the seedlings with *Trichoderma harzianum* (14.32 cm). While maximum breadth of leaves (16.58 cm) was found on treating the seedling with *Trichoderma viride* during 2017-18. The data pertaining in Tables 11 & 12 indicates that among all the treatment T<sub>1</sub> and T<sub>2</sub> are statistically at par with each other. However, it was found that T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> are non-significant with each other. Among pooled data it was found that maximum breadth of leaves was observed on treating the plant with *Trichoderma harzianum*. Hyakumachi and Kubota [15] describe plant growth-promoting fungi (PGPF) as a microorganism that can stimulate plant growth. The major impacts of these PGPF are commonly shown on the crop growth, quality of the final yield and productivity. Recently, researches have revealed that *Trichoderma* spp. can be an excellent PGPF.

**Table 11. Effect of different treatments on Leaf length (cm) of betelvine during 2016-2017, 2017-2018 and pooled**

Treatments		1st year	2nd year	Pooled
T <sub>0</sub>	Control (Inoculated)	7.81	6.05	6.93
T <sub>1</sub>	Copper oxychloride	14.88c	15.46b	15.17b
T <sub>2</sub>	<i>T. harzianum</i>	14.23c	17.42	15.83b
T <sub>3</sub>	<i>T. viride</i>	14.25c	15.86b	15.06b
T <sub>4</sub>	<i>Bacillus subtilis</i>	11.77a	11.96a	11.87a
T <sub>5</sub>	Neem oil	11.57ab	11.88a	11.73a
T <sub>6</sub>	Tobacco leaf extract	10.91a	10.40	10.66
S. Ed. (±)		0.375	0.460	0.417
C. D. (P = 0.05)		0.794	0.976	0.885

\*The figures followed by the same letters in the column are non-significant with each other

**Table 12. Leaf breadth (cm) of betelvine as affected by different treatments**

Treatment	Treatment Details	1st year	2nd year	Pooled
T <sub>0</sub>	Control (Inoculated)	7.48	6.37	6.93
T <sub>1</sub>	Copper oxychloride	14.98b	14.21b	14.60b
T <sub>2</sub>	<i>T. harzianum</i>	14.32b	15.41b	14.87b
T <sub>3</sub>	<i>T. viride</i>	11.77a	16.58bc	14.18b
T <sub>4</sub>	<i>Bacillus subtilis</i>	14.47b	9.28a	11.88a
T <sub>5</sub>	Neem oil	11.57a	10.15a	10.86a
T <sub>6</sub>	Tobacco leaf extract	11.16a	10.18a	10.67a
S. Ed. (±)		0.633	0.615	0.601
C. D. (P = 0.05)		1.341	1.303	1.274

Similar result was also noticed by Dasgupta et al. [16] that *Trichoderma* application with MOC at quarterly interval increases the yield as compare to control and check Copper oxychloride. This trend was noticed in both the years and also in pooled mean. Also, in case of Neem oil is usually used as an insecticide but also has fungicide action. Gurjar et al. [17]. Neem oil foliar spray after initiation of disease symptoms in order to have a higher profitable yield and higher economic return without health risk as well as environmental pollution [18].

#### 4. CONCLUSION

Among all the treatments the maximum leaf length was recorded in Copper oxychloride (14.88), and minimum control (Inoculated) (7.81), in 1<sup>st</sup> year (2016-17). However, in 2<sup>nd</sup> year (2017-2018), the maximum leaf length was *T. harzianum* (17.42), control (Inoculated) (6.05). In Pooled data the maximum leaf length in *T. harzianum* (15.83), the leaf length was lowest in control (Inoculated) (6.93). Among all the treatments the maximum leaf breadth was recorded in Copper oxychloride (14.98), and minimum control (Inoculated) (7.48), in 1<sup>st</sup> year (2016-17). However, in 2<sup>nd</sup> year the maximum leaf breadth was *T. viride* (16.58), and control (Inoculated) (6.37). In Pooled data the maximum leaf breadth in *T. harzianum* (14.87), the leaf breadth was lowest in control (Inoculated) (6.93).

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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