Original Research Article

Potential of Bioagent and botanicals on vegetavive growth parameter of betelvine against leaf spot caused by *Colletotrichum capcisi*

Abstract

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

Key words: *Trichoderma harzianum, Bacillus subtallis*, Anthracanose, *Colletotrichum capsici*

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

Comment [U1]: Use vegetative not vegetavive

Comment [U2]: Use energetic instead energestic

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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%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 **Chopra *et al.* (1956).** 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.*(1956)**. 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 **Chu (2001); Garg and Jain (1996**). The most probable place of origin of the betelvine is Malaysia **Chattopadhyay and Maity (1967).** 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 X*anthomonas 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.* (1987)** that can kill plants and produce up to 25-90% of the crop in different parts of India **Dastur (1935); Chattopadhayay and Maiti (1990)**. This disease of betelvine was first identified by (Roy, 1948) and it is also known as anthracnose of 'Pan' (vernacularly called as Jhalma').

Comment [U7]: Use energetic instead energestic

Comment [G8]: This sentence contains negative information and you cannot talk about a production of 20-90% ("produce up to 25-90%")I suppose the correct is "a reduction of 20-90%" as a result of *Colletotrichum capsici* attacks.

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. 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^{o}C during summers and goes down to 10-23^{o}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 (Ciju, 2013). After four months of planting, more soil (Gasti) and bamboo stick (washi) was added. It was done again at two months of intervals.

Result and Discussion

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. 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 during 2016-17 and 78.40 during 2017-18 with mean value 66.95 at 60 DAP was observed in T_2 treatment which was closely followed by T_3 treatment exhibited 53.00 and 78.05 with mean value 65.53 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) followed by *Trichoderma viride* (73.65). 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 herzianum* which was statistically at par with *Trichoderma viridae* (T_3). As the length of stem increases, number of leaves increases thereby economic importance of crop as well as

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economic benefit of farmer will increases. Calvo *et al.* (2010) 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 (Contreras-Cornejo *et al*., 2015a, Contreras-Cornejo *et al*., 2015b; *Kubicek et al.*, 2001; Kullnig *et al.*, 2000). Similar result D'Souza et al. (2001) reported significant reduction of disease with the use of an isolate of *Trichoderma harzianum*.

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Comment [G12]: As this is the first time this species is mentioned, please write the full name, *Rhizoctonia*

Table 1 : Effect of different treatments on plant height (cm) at 60 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_0	Control (Inoculated)	35.40	48.35	**41.88**
T_1	Copper oxychloride	50.30	66.85	**58.58**
T_2	*T. harzianum*	55.50	78.40b	**66.95a**
T_3	*T. viride*	53.00	78.05b	**65.53a**
T_4	*Bacillus subtilis*	47.30	62.45a	**54.88**
T_5	Neem oil	43.85	60.95a	**52.40**
T_6	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 Notation	Treatment Details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T_0	Control (Inoculated)	49.60	55.75	**52.68**
T_1	Copper oxychloride	64.40	81.45	**72.93**
T_2	*T. harzianum*	76.75	96.85	**86.80**
T_3	*T. viride*	73.65	91.25	**82.45**
T_4	*Bacillus subtilis*	60.75	78.65a	**69.70**
T_5	Neem oil	58.25	76.45a	**67.35**
T_6	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 Notation	Treatment Details	1st year (in cm)	2nd year (in cm)	Pooled (in cm)
T_0	Control (Inoculated)	63.55	58.45	**61.00**
T_1	Copper oxychloride	77.65a	90.95b	**84.30a**
T_2	*T. harzianum*	91.90b	115.20	**103.55b**
T_3	*T. viride*	91.10b	112.25	**101.68b**
T_4	*Bacillus subtilis*	75.25a	79.30a	**77.28**
T_5	Neem oil	74.50a	89.10b	**81.80a**
T_6	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_0	Control (Inoculated)	71.35	91.05	**81.20**
T_1	Copper oxychloride	90.10b	125.90	**108.00**
T_2	*T. harzianum*	110.45c	151.05a	**130.75b**
T_3	*T. viride*	108.40c	148.75a	**128.58b**
T_4	*Bacillus subtilis*	88.70ab	119.05	**103.88a**
T_5	Neem oil	86.45a	115.40	**100.93a**
T_6	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_0	Control (Inoculated)	91.45	117.90	**104.68**
T_1	Copper oxychloride	120.25	170.15	**145.20a**
T_2	*T. harzianum*	142.90b	159.35a	**151.13**
T_3	*T. viride*	141.60b	153.00	**147.30a**
T_4	*Bacillus subtilis*	114.00a	166.30	**140.15**
T_5	Neem oil	109.60a	161.05a	**135.33**
T_6	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	Treatment Details	1st year (in	2nd year	Pooled (in

Notation		cm)	(in cm)	cm)
T_0	Control (Inoculated)	121.90	149.15	**135.53**
T_1	Copper oxychloride	165.40	229.85	**197.63**
T_2	*T. harzianum*	188.00a	268.25	**228.13**
T_3	*T. viride*	186.30a	260.10	**223.20**
T_4	*Bacillus subtilis*	156.65	220.10	**188.38**
T_5	Neem oil	151.25	207.60	**179.43**
T_6	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_0	Control (Inoculated)	155.25	181.25	**168.25**
T_1	Copper oxychloride	224.30	259.10a	**241.70a**
T_2	*T. harzianum*	257.70	280.20b	**268.95**
T_3	*T. viride*	250.65	276.45b	**263.55**
T_4	*Bacillus subtilis*	216.00	260.55a	**238.28a**
T_5	Neem oil	205.30	242.95	**224.13**
T_6	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_0	Control (Inoculated)	188.05	191.70	**189.88**
T_1	Copper oxychloride	264.65	299.30	**281.98**
T_2	*T. harzianum*	272.60a	315.35	**293.98**
T_3	*T. viride*	268.05a	308.60	**288.33**
T_4	*Bacillus subtilis*	251.95	285.30	**268.63**
T_5	Neem oil	246.35	261.20	**253.78**
T_6	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	Treatment Details	1st year (in	2nd year	Pooled (in

Notation		cm)	(in cm)	cm)
T_0	Control (Inoculated)	198.80	200.75	**199.78**
T_1	Copper oxychloride	295.20	317.9	**306.55**
T_2	*T. harzianum*	310.25	340.15	**325.20**
T_3	*T. viride*	303.6	331.30	**317.45**
T_4	*Bacillus subtilis*	271.65	301.70	**286.68**
T_5	Neem oil	260.85	276.60	**268.73**
T_6	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_0	Control (Inoculated)	209.85	200.66	**205.206**
T_1	Copper oxychloride	315.30a	317.86	**316.58**
T_2	*T. harzianum*	324.90b	339.86	**332.38**
T_3	*T. viride*	320.45ab	331.40	**325.93**
T_4	*Bacillus subtilis*	285.10	301.60	**293.35**
T_5	Neem oil	271.40	276.53	**273.97**
T_6	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**

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, T1, T2 and T3 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 herzianum*.

Comment [U13]: Use *harzianum* instead *herzianum*

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_0	Control (Inoculated)	7.81	6.05	**6.93**

T_1	Copper oxychloride	14.88c	15.46b	**15.17b**
T_2	*T. harzianum*	14.23c	17.42	**15.83b**
T_3	*T. viride*	14.25c	15.86b	**15.06b**
T_4	*Bacillus subtilis*	11.77a	11.96a	**11.87a**
T_5	Neem oil	11.57ab	11.88a	**11.73a**
T_6	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.

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_1 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_2 treatment *i.e* treating the seedlings with *Trichoderma herzianum* (14.32 cm). While maximum breadth of leaves (16.58 cm) was found on treating the seedling with *Trichoderma viridae* during 2017-18. The data pertaining in table indicates that among all the treatment T_1 and T_2 are statistically at par with each other. However, it was found that T_4, T_5 and T_6 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 herzianum*. Hyakumachi and Kubota (2003) 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. Similar result was also noticed by Dasgupta *et al.* (2003) 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., (2012). 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.

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

Comment [U14]: Use *harzianum* instead *herzianum*

Comment [U15]: Use *harzianum* instead *herzianum*

Comment [G16]: These three sentences are identical with **Zin, N. A. and Badaluddin, N. A. (2020).** Biological functions of *Trichoderma* spp. for agriculture applications. *Annals of Agricultural Sciences*; **65**: 168–178. Please, adapt the sentences and include the mentioned reference.

Treatment	Treatment Details	1st year	2nd year	Pooled
T_0	Control (Inoculated)	7.48	6.37	6.93
T_1	Copper oxychloride	14.98b	14.21b	14.60b
T_2	*T. harzianum*	14.32b	15.41b	14.87b
T_3	*T. viride*	11.77a	16.58bc	14.18b
T_4	*Bacillus subtilis*	14.47b	9.28a	11.88a
T_5	Neem oil	11.57a	10.15a	10.86a
T_6	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**

Conclusion

Among all the treatments the maximum leaf length was recorded in Copper oxychloride (14.88), and minimum control (Inoculated) (7.81), in 1st year (2016-17). However, in 2nd 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 1st year (2016-17). However, in 2nd 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).

Comment [G17]: Insert “at” or “in”

Comment [G18]: compared with

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