### Original Research Article

Synergistic effects of seed inoculation with *Piriformospora indica* and foliar hormonal application on early flowering in rice bean(*Vigna umbellata*) variety Surabhi

#### **ABSTRACT**

This study aimed to evaluate the effectiveness of seed inoculation with *Piriformospora indica* and various foliar hormonal treatments in promoting early flowering in fodder rice bean (*Vigna umbellata* Thunb.), variety Surabhi, conducted at the College of Agriculture, Vellayani. The experiment was laid out in randomized block design with ten treatments and three replications. The plot size of 3 m² was followed in the field experiment.

The experiment assessed the impact of different treatments on flowering and other biometric traits in the leguminous crop. The treatments included untreated control, seed inoculation with *Piriformospora indica* before sowing and foliar applications of salicylic acid (100 ppm,150 ppm), GA<sub>3</sub> (200 ppm,300 ppm), Paclobutrazol (10 ppm,20 ppm) and KNO<sub>3</sub> (1%,1.5%) at 30 days after sowing (DAS). The results revealed that significant differences were observed among treatments for all thirteen biometric traits measured. Early flowering was recorded in plants treated with salicylic acid 0 100 ppm(65.35 days), followed by GA<sub>3</sub> 0 300 ppm (67.72 days), salicylic acid 150 ppm (67.81 days), and Paclobutrazol 10 ppm (69.87 days), whereas the control flowered last (80.96 days. The findings suggest that the application of specific concentrations of growth regulators and seed inoculation with *P.indica* can effectively reduce the time to flowering, enhancing the potential for improved seed production in fodder rice bean.

Keywords: Fodder rice bean, Piriformospora indica, early flowering, salicylic acid.

#### 1. INTRODUCTION

Rice bean (*Vigna umbellata*) is an often-overlooked legume, regarded as a minor food and fodder crop in Northern and North-eastern India. It is grown on 20,000 hectares in India, yielding an average green fodder productivity of 15-30 tons per hectare. Its cultivation is primarily limited to the tribal areas of the North-eastern hills and the hilly regions of the Western and Eastern ghats.

Fodder rice bean is an excellent protein source, containing 20-25 percent protein on a dry weight basis, making it ideal for livestock, especially dairy cattle that need high protein levels for milk production. Besides protein, it is also rich in vitamins and minerals like vitamin A, vitamin B, iron, and calcium, contributing to the health and productivity of livestock [1].

Rice bean thrives well in Kerala's climate, according to a study by Bhoomika [2] from Kerala Agricultural University. However, issues were observed with flowering and seed setting. Since rice bean is propagated by seeds, hence, proper flowering and seed setting are crucial for producing high-quality seeds.

KAU released a fodder rice bean variety Surabhi in 2016. However, seed production for this variety has been inconsistent due to limited flowering. Applying exogenous hormones has been shown to successfully regulate flowering and improve seed set in various crops. This study aimed to identify effective hormonal treatments to enhance flowering and seed set in rice bean.

The livestock sector is a crucial part of the Indian economy and an essential component of Indian agriculture, providing livelihood support to more than two-thirds of the rural population and serving as the primary income source for small and marginal farmers. However, this sector struggles with a shortage of feed and fodder, a challenge that can be addressed through the cultivation of high-quality fodder crops. Recently, leguminous crops have gained attention as nutritious fodder for animals, with rice bean, a protein-rich legume, recognized for its role in enhancing milk production in livestock. However, flowering and subsequently seed set were found to be erroneous which impedes the demand for quality seeds of high yielding varieties of this crop.

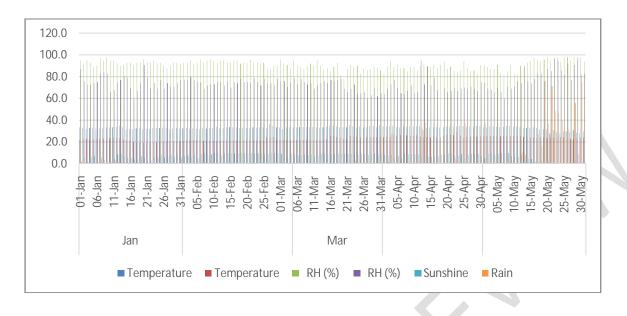
#### 2. MATERIALS AND METHODS

#### 2.1.Plant materials and methods

The experimental material for the present study was fodder rice bean ( $Vigna\ umbellata\ (Thunb)$ ) variety Surabhi from KAU. The treatments included untreated control, seed inoculation with  $Piriformospora\ indica$  before sowing and foliar applications of salicylic acid (100 ppm; 150 ppm), GA<sub>3</sub> (200 ppm; 300 ppm), Paclobutrazol (10 ppm; 20 ppm) and KNO<sub>3</sub> (1%; 1.5%) at 30 days after sowing (DAS). The research area was divided into 3 blocks, each block was subdivided into ten plots, and size of each plot was 3m × 2m (6.0 m²). The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

#### 2.2. Weather conditions

The weather data on monthly average temperature, relative humidity and rainfall during research period were recorded regularly by the official website system of the Kerala Agricultural University( <u>Directorate of Information Systems (DIS)</u>, <u>K.A.U.</u>, <u>Thrissur</u>). The data regarding temperature (minimum-maximum, and mean), relative humidity and rainfall during the experimentation are presented in **Figure 1**.



**Figure 1:** Monthly average temperature (minimum, maximum, and mean), relative humidity (%), and rainfall (mm) during the experiment.

# 2.3. Analysis of variance(ANOVA)

# 2.3.1. Analysis in Randomized Block Design (RBD)

The randomized block design (RBD) was adopted with three replications. As per the method given by Panse and Sukhatme[3]the analysis of variance was carried out.

Total variation for each character is portioned into variation due to treatments, blocks and error.

 $Y_{ij}=m + g_i + e_{ij}$ 

Where.

 $Y_{ij}$ = Phenotypic observation of  $i^{th}$  genotype in  $j^{th}$  replication

m = General mean

 $g_i$  = True effect of  $i^{th}$  genotype

rj = True effect of j<sup>th</sup> replication

e ij=Random error associated with i<sup>th</sup> genotypes in j<sup>th</sup> replication

Based on above model analysis of Variance (ANOVA)was carried out for all the characters.

Source of	Df	SS	MSS	F-ratio
variation				
Replication	r-1	RSS	M <sub>sr</sub>	$M_{sr}/M_{e}$
Treatments	t-1	TSS	M <sub>st</sub>	M <sub>st</sub> /M <sub>e</sub>
Error	(r-1)(t-1)	ESS	M <sub>se</sub>	
Total	rt-1			

# Where.

r = Number of replications

t = Number of treatments

 $M_{sr}$  = Mean sum of squares of replications

 $M_{st}$  = Mean sum of squares of replications

M<sub>se</sub>=Mean sum of squares of error

Df =Degrees of freedom

The significance of mean sum of squares for each character was tested against the corresponding error degrees of freedom using 'F' test(Fisher and Yates[4]).

Range, mean, standard error of mean, critical difference and co-efficient of variation were calculated to study components of variance among genotype.

Range = Highest value-Lowest value

Standard Error Mean,

$$(SE (m)) = (Mse/r)^{1/2}$$

Where,

M<sub>se</sub>=Error mean squares

R= Number of replications

Critical difference.

 $C.D=S.E(d) \times t$ 

Where,

Standard error,

S.E(d)=
$$(^{Mse}/_r)^{1/2}$$

't' =t table value at error degrees of freedom

Co-efficient of variation,

C.V=
$$(S.D/\bar{X})$$
 x 100

Where, S.D=Standard deviation of the population

 $\bar{X}$  =Population mean

# 3. RESULTS AND DISCUSSION

# 3.1.ANOVA

The analysis of variance revealed that mean sum of squares due to treatments was significant for all the characters.

Table1: Analysis of variance for 14 characters in fodder rice bean, var. Surabhi

SI.No.	Characters		are	
		Replication	Treatment	Error
1	Leaf area index	0.053	0.053*	0.014
2	Number of branches	0.054	0.568*	0.182
3	Plant height(cm)	46.368	457.885*	48.627
4	Days to first flowering	23.593	95.916*	2.047
5	Days to 50 % flowering	24.771	116.356*	5.276
6	Number of pods per plant	13.528	41.341*	13.523
7	Days to maturity	159.612	80.553*	23.944
8	100 seeds weight(g)	0.756	1.302*	0.423
9	Number of seeds per plant	911.322	1784.331*	693.445
10	Seeds yield per plant <mark>(g)</mark>	12.395	34.538*	13.826
11	Seeds yield per plot(g)	84.792	607.042*	61.752
12	Green fodder yield per plot(t ha-1)	2.94	82.856*	1.022
13	Dry fodder yield per plot(t ha <sup>-1</sup> )	0.173	3.272*	0.623

<sup>\* :</sup> significant at p ≤ 0.05

Table 2:Mean performance of various characters in rice bean(var.Surabhi)

Treatments	LAI	NB	HT	DF	DFF	PP	SPP	SYPP	SYPPL	100 SW	GFY	DFY	DM
Control(T₁)	4.18 <sup>e</sup>	4.03 <sup>d</sup>	104.20 <sup>cd</sup>	80.96 <sup>a</sup>	92.57 <sup>a</sup>	21.20 <sup>d</sup>	139.40	19.70 <sup>d</sup>	63.36 <sup>b</sup>	5.56 <sup>a</sup>	16.94 <sup>d</sup>	4.69 <sup>bc</sup>	109.67 <sup>ab</sup>
Piriformospora indica(T₂)	6.36 <sup>a</sup>	5.39 <sup>a</sup>	129.90 <sup>a</sup>	73.40 <sup>c</sup>	83.20 <sup>cd</sup>	32.62 <sup>a</sup>	214.36	29.61 <sup>a</sup>	81.88 <sup>a</sup>	5.03 <sup>a</sup>	29.16 <sup>a</sup>	7.85 <sup>a</sup>	103.73 <sup>bc</sup>
Salicylic acid 100ppm (T <sub>3</sub> )	4.42 <sup>d</sup>	5.01 <sup>abc</sup>	107.73 <sup>bc</sup>	65.36 <sup>f</sup>	74.80 <sup>f</sup>	27.00 <sup>abc</sup>	187.72 abc	25.93 <sup>a</sup> bcd	84.38 <sup>a</sup>	3.89 <sup>b</sup>	17.07 <sup>d</sup>	4.67 <sup>bc</sup>	102.83 <sup>bc</sup>
Salicylic acid 150ppm (T <sub>4</sub> )	4.05 <sup>ef</sup>	5.13 <sup>ab</sup>	104.07 <sup>cd</sup>	67.81 <sup>def</sup>	76.84 <sup>ef</sup>	24.30 <sup>bcd</sup>	165.27 bcd	22.87 <sup>b</sup>	47.88 <sup>c</sup>	5.21 <sup>a</sup>	16.87 <sup>d</sup>	4.46 <sup>bc</sup>	99.20°
GA <sub>3</sub> 200ppm (T <sub>5</sub> )	6.18 <sup>a</sup>	5.30 <sup>ab</sup>	124.74 <sup>a</sup>	70.23 <sup>d</sup>	79.47 <sup>de</sup>	23.56 <sup>bcd</sup>	161.14 bcd	21.93 <sup>c</sup>	55.09 <sup>bc</sup>	4.80 <sup>ab</sup>	26.95 <sup>b</sup>	5.81 <sup>b</sup>	104.43 <sup>bc</sup>
GA <sub>3</sub> 300ppm(T <sub>6</sub> )	6.25 <sup>a</sup>	5.02 <sup>abc</sup>	127.50 <sup>a</sup>	67.72 <sup>ef</sup>	77.80 <sup>ef</sup>	23.73 <sup>bcd</sup>	164.97 bcd	22.63 <sup>b</sup>	62.89 <sup>b</sup>	4.58 <sup>ab</sup>	28.08 <sup>a</sup>	5.81 <sup>b</sup>	99.25°
Paclobutrazol 10 ppm (T <sub>7</sub> )	4.12 <sup>ef</sup>	4.64 <sup>bcd</sup>	102.63 <sup>cd</sup>	69.87 <sup>de</sup>	80.61 <sup>de</sup>	28.43 <sup>abc</sup>	195.39	27.43 <sup>a</sup>	85.34 <sup>a</sup>	5.32 <sup>a</sup>	16.75 <sup>d</sup>	4.62 <sup>bc</sup>	100.83°
Paclobutrazol 20 ppm (T <sub>8</sub> )	3.93 <sup>f</sup>	4.37 <sup>cd</sup>	93.43 <sup>d</sup>	78.79 <sup>ab</sup>	91.94 <sup>a</sup>	29.68 <sup>ab</sup>	199.97	28.53 <sup>a</sup>	64.09 <sup>b</sup>	3.75 <sup>b</sup>	15.86 <sup>d</sup>	4.42 <sup>c</sup>	114.57 <sup>a</sup>
KNO <sub>3</sub> 1%( (T <sub>9</sub> )	4.71 <sup>c</sup>	5.25 <sup>ab</sup>	118.45 <sup>ab</sup>	78.11 <sup>b</sup>	84.64 <sup>bc</sup>	22.20 <sup>cd</sup>	150.03	21.00 <sup>d</sup>	47.13 <sup>c</sup>	4.65 <sup>ab</sup>	19.14 <sup>c</sup>	5.27 <sup>bc</sup>	110.91 <sup>ab</sup>
KNO <sub>3</sub> 1.5%(T <sub>10</sub> )	5.01 <sup>b</sup>	4.97 <sup>abc</sup>	118.94 <sup>ab</sup>	78.83 <sup>ab</sup>	87.93 <sup>b</sup>	22.80 <sup>cd</sup>	156.33	22.70 <sup>b</sup>	59.67 <sup>bc</sup>	3.78 <sup>b</sup>	20.43 <sup>c</sup>	5.59 <sup>bc</sup>	106.81 <sup>abc</sup>
S.E.	0.069	0.247	4.026	0.826	1.326	2.123	15.204	2.147	4.537	0.376	0.584	0.456	2.825
C.D.(5%)	0.205	0.733	11.962	2.454	3.940	6.308	45.172	6.378	13.480	1.116	1.734	1.354	8.394

LAI-Leaf area index
NB-Number of branches
HT-Plant height
DF-days to first flowering
DFF-Days to 50% flowering

PP-Number of pods per plant SPP-Number of seeds per plant SYPP-Seeds yield per plant SYPPL-Seeds yield per plot 100 SW-100 seeds weight GFY-Green fodder yield per plot DFY-Dry fodder yield per plot DM-Days to maturity

# 3.2. Mean performance of various characters in rice bean(var.Surabhi)

The mean performance of fodder rice bean(Var.Surabhi)in response to different treatments for all the characters studied are presented in the Table 2.

### 3.2.1.Leaf area index (LAI)

The leaf area index (LAI) varied with treatments and the maximum LAI recorded by the treatment P. indica (6.36) which was on par with  $GA_3$  @ 300 ppm (6.25) and  $GA_3$  @ ppm(6.18) while the minimum LAI was observed in treatments with Paclobutrazol @ 20 ppm (3.93).

#### 3.2.2. Number of branches

Treatment with *P. indica* (5.39) exhibited the highest number of branches, followed by GA<sub>3</sub> @ 200 ppm (5.30) which was on par with KNO<sub>3</sub> 1% (5.25) while the lowest number was observed in the control (4.03).

### 3.2.3.Plant height(cm)

The highest plant height was observed in plants treated with *P. indica* (129.9 cm), which was on par with GA<sub>3</sub> @ 300 ppm (127.5 cm) and GA<sub>3</sub> @ 200 ppm (124.74 cm), while the lowest plant height was recorded in treatments with Paclobutrazol @ 20 ppm (93.43 cm).

# 3.2.4.Days to first flowering

Early flowering was observed in SA <a>@</a> 100ppm treated plants (65.36 days), followed by GA<sub>3</sub> <a>@</a> 300 ppm (67.72 days), SA <a>@</a> 150 ppm (67.81 days), and Paclobutrazol <a>@</a> 10 ppm (69.87 days), while late flowering was recorded in the control (80.96 days), with a mean performance of 73.10 days.

### 3.2.5. Days to 50 percent flowering

The treatment with SA @ 100 ppm took minimum number of days to attain 50 per cent flowering (74.8 days), while the maximum number of days was observed in the control (92.57 days) which was on par with Paclobutrazol 20ppm(91.94 days) with a mean of 82.98 days.

## 3.2.6. Number of pods per plant

The highest number of pods per plant was observed in the treatment with *P. indica* (32.62), followed by Paclobutrazol 20 ppm (29.68) and Paclobutrazol 0 10 ppm (28.43). The minimum number of pods was recorded in the control (21.2), followed by KNO<sub>3</sub> 1 1% (22.2) which was on par with KNO<sub>3</sub> 1.5% (22.8), with a mean value of 25.55.

## 3.2.7.100 seeds weight(g)

The highest 100-seed weight was observed in the control (5.56g)which was on par with Paclobutrazol @ 10 ppm (5.32g) ,SA @ 150 ppm (5.21g) and *P.indica*(5.03) while the lowest was recorded for Paclobutrazol @ 20 ppm (3.75g) which was on par with KNO<sub>3</sub> @ 1.5% (3.78g), with a mean value of 4.66g.

#### 3.2.8. Seeds per plant

The highest number of seeds per plant was recorded in the treatment with *P. indica* (214.36), followed by Paclobutrazol © 20 ppm (199.97) which was on par with Paclobutrazol © 10 ppm (195.39). The lowest number of seeds per plant was observed in the control (139.4), followed by KNO<sub>3</sub> © 1% (150.03) and KNO<sub>3</sub> © 1.5% (156.33), with a mean value of 173.46.

## 3.2.9.Seed yield per plant(g)

The seed yield per plant was recorded the highest for plants treated with in *P. indica* (29.61 g), followed by Paclobutrazol @ 20 ppm (28.53 g). The lowest seed yield per plant was observed in the control (19.70 g)which was on par with KNO<sub>3</sub> @ 1% (21 g), with a mean value of 24.23 g.

# 3.2.10.Seed yield per plot(g)

The highest seed yield per plot was recorded in Paclobutrazol at 10 ppm (85.34 g)which was on par with SA @ 100 ppm (84.38 g) and *P. indica* (81.88 g), while the lowest yields were observed in KNO<sub>3</sub> @ 1% (47.13 g)which was on par with SA @ 150 ppm (47.88 g) followed by GA<sub>3</sub> @ 200 ppm (47.89 g) with a mean value of 65.17 g.

# 3.2.11.Green fodder yield (t ha<sup>-1</sup>)

The maximum green fodder yield was recorded in *P. indica* (29.16 t ha<sup>-1</sup>), followed by GA<sub>3</sub> @ 300 ppm (28.08 t ha<sup>-1</sup>) and GA<sub>3</sub> @ 200 ppm (26.95 t ha<sup>-1</sup>). The minimum green fodder yield was observed in Paclobutrazol @ 10 ppm (16.75 t ha<sup>-1</sup>) which was on par with SA @ 150 ppm (16.87 t ha<sup>-1</sup>) and the control (16.95 t ha<sup>-1</sup>), with a mean value of 20.72 t ha<sup>-1</sup>.

#### 3.2.12.Dry fodder yield per plot(t ha<sup>-1</sup>)

The highest dry fodder yield was recorded in *P. indica* (7.85 t ha<sup>-1</sup>), followed by GA<sub>3</sub> @ 200 ppm and GA<sub>3</sub> @ 300 ppm

(5.81 t ha<sup>-1</sup>). The lowest dry fodder yield was observed in Paclobutrazol @ 20 ppm (4.42 t ha<sup>-1</sup>), followed by SA @ 150 ppm (4.46 t ha<sup>-1</sup>) which was on par with SA @ 100 ppm (4.67 t ha<sup>-1</sup>), with a mean value of 5.32 t ha<sup>-1</sup>.

## 3.1.13. Days to maturity

Days to maturity among the treatments varied from 99.2 days to 114.57 days. Highest number of days to mature was taken by Paclobutrazol @ 20 ppm(114.57 days) followed by KNO<sub>3</sub> 1%(110.91 days), whereas SA @ 150 ppm(99.2 days)matured first followed by GA<sub>3</sub> @ 300 ppm(99.25 days)

In the experiment the thirteen characters studied for ten different treatments showed considerable variation among treatments. The ANOVA indicated that mean sum of squares due to treatments were highly significant for the characters leaf area index, number of branches, plant height, days to first flowering, days to 50% flowering, number of pods per plant, days to maturity,100 seeds weight, number of seeds per plant, seeds yield per plant, seeds yield per plot, green fodder yield per plot, and dry fodder yield per plot. Similar result was obtained by Fikreselassie and Seboka[5]in faba bean and Gupta[6] in rice bean,.

Mean performance of thirteen traits in the treatments indicated significant differences for all the traits among treatments. Leaf area index is the ratio of projected area of leaves over unit of land and it is the measure of the active photosynthetic area. Higher LAI denotes higher fodder yield production. Leaf area index ranged from 3.93(Paclobutrazol @ 20 ppm) to 6.36(*P.indica*). In fodder rice bean LAI between 2.81 to 4.06 was reported in by Aswathy [7] and from 2.15 to 4.64 was reported by Bhoomika [2]. Leaf area index of 6.79 was recorded by Ajmal [8] in fodder rice bean. Highest number of branches was observed in plants treated with *P.indica*(5.39) and lowest were noted in untreated control(4.03). In rice bean, Ahirwal [9] reported mean value of 5.85 for number of branches. Numbers of branches varied from 4.8 to 8.9 in a study conducted by Neelam *et al.* [10] in fodder horse gram.

In the present study, plant height varied from 93.43 cm (Paclobutrazol @ 20 ppm) to 129.0 cm (*P.indica*). Plant height ranging from 84.21 cm to 264.08 cm was obtained by Kujur [11]; from 85.56 cm to 143.89 cm were observed in a study by Ajmal [8]and from 101.23 cm to 145.06 cm by Bhoomika [2] in fodder rice bean. Number of pods per plant ranged from 21.20(control) to 32.62(*P.indica*). A mean of 32.33 pods per plant was recorded in a study on rice bean by Kumar and Elamathi [12]. In the study by Prashant [13] in fodder rice bean, pods per plant ranged from 16.37 to 21.27. Green fodder yield per plot ranged from 15.86 t ha (Paclobutrazol 20 ppm) to 29.16 t ha (*P.indica*). Green fodder yield of about 81 gram per plant to 167 gram per plant was obtained by Jharia [14] study conducted in rice bean. Bhoomika [2] reported green fodder yield between 22.32 t ha to 35.49 t ha and Ajmal [8] recorded a range of 10.20 to 17.29 t ha similar trend was followed in dry fodder yield per plot.

In this experiment, among the treatments evaluated, plants treated with SA @ 100 ppm exhibited the earliest flowering (65.36 days), followed by GA<sub>3</sub> @ 300 ppm,SA @ 150 ppm (67.81 days) and the Paclobutrazol @ 10 ppm (69.87 days). Similar effects were observed by Nicu *et al.* [15] in *Zinnia elegans*. Regarding GA<sub>3</sub> application, early flowering was noted in plants treated with GA<sub>3</sub> @ 300 ppm (67.72 days) and GA<sub>3</sub> @ 200 ppm (70.23 days) compared to the control (80.96 days), which is consistent with the findings of Mujadid *et al.* [16] in *Tagetes erecta*.

Paclobutrazol @ 10 ppm treated plants flowered in 69.87 days, followed by 20 ppm (78.79 days), while the control flowered later, contradicting the results reported by Bably *et al.* [17] in *Jacobinia carnea*. For KNO<sub>3</sub> treatments, plants treated with 1% KNO<sub>3</sub> flowered earlier (78.11 days) than those treated with 1.5% (78.83 days), with control plants flowering last, in agreement with the conclusions of Padmalatha *et al* [18]. Furthermore, rice bean inoculated with *Piriformospora indica* exhibited earlier flowering (73.4 days) compared to control plants (80.96 days), which aligns with the findings of Pan *et al.* [19].

Overall, the use of plant growth regulators and inoculants effectively promoted earlier flowering across various treatments, highlighting their potential in regulating phenological events in crop plants.

#### 4. CONCLUSION

This study demonstrated that the seed inoculation with *Piriformospora indica* as well as foliar application of salicylic acid, gibberellic acid, Paclobutrazol, and KNO<sub>3</sub> at 30 DAS had a significant positive impact on early flowering in fodder rice bean plants compared to the control. The most pronounced effect on early flowering was observed with salicylic acid at 100 ppm, while untreated plants exhibited the least response. These findings suggest that the flowering time of fodder rice bean can be effectively manipulated through exogenous foliar applications of growth regulators and seed inoculation with *Piriformospora indica*, which could enhance seed production efficiency in fodder rice bean cultivation.

#### **6.COMPETITIVE INTERESTS**

Authors have declared that no competing interests exists.

Disclaimer (Artificial intelligence)

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