Powdery Mildew (Erysiphe Pisi) Management Studies in Grass pea (Lathyrus Sativus L)

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Abstract
Present investigations were undertaken to screen Grass pea germplasm for their resistance against powdery mildew in field condition & to test the efficacy of fungicides in vitro. The sixty Grass pea genotypes screened under natural field condition for the disease reaction against powdery mildew. The Experimental results revealed that elven genotypes including check RLK-279 were highly resistant, while twenty-four showed resistant reaction, sixteen were moderately resistant, and nine were moderately susceptible including susceptible check Bio R-231 and none of the genotypes has shown susceptible and highly susceptible reaction (SR). The in-vitro studies revealed that, Tridemorph or Hexaconazole has inhibited the growth of E. pisi significantly. Thus, spraying of Tridemorph significantly managed powdery mildew disease caused by E. pisi in Grass pea with yield enhancement.

Introduction
Grass pea (Lathyrus sativus L.) is an important pulse crop. It is commonly known as Khesari or Chickling pea or Chickling vetch or Blue vetching or Teora and Lakh-Lakhdi. This crop is mainly grown in India, Pakistan, Bangladesh, Nepal, and South America and in different regions of African countries.

Grass pea is considered as a native of Europe and West Asia. The Grass peas third largest pulse crop of India with respect to acreage (1.5 million ha.) after chickpea and pigeon pea, having a production of nearly 0.8 million tones and productivity of 559 kg/ha.

The Grass pea is a self-fertilized legume crop with diploid species (2n = 14). The Grass pea plant has branches which are sub - erect straggling or climbing herbaceous winter annual; stem grows to height of 60-90 cm tall; leaves are pinnately compound, with rachis ending in a tendril and stipule pair. Flowers are solitary auxiliary, papilionaceous having reddish purple, pink, blue or white colors. The pods are oblong, slightly curved and dehisce after maturity. The seeds are white, grayish-brown or yellowish in color usually spotted or mottled. Usually one pod contains 3-5 seeds.
The Grass pea plant has shown remarkable tolerance to adverse environmental conditions such as drought and water logging up to seed germination. However, highly susceptible to water logging during growth period. This hardy crop can be grown in all types of soils except highly acidic ones. The Grass pea crop can be successfully grown on lands which are considered unfit for other pulses and cereal crops. The Grass pea has reported to be tolerant to annual precipitation of 32-136 cm, annual mean temperature of 4.32 – 27.5°C and pH of 4.5 – 8.3. In India Grass pea extensively cultivated in the plains as well as in the hilly slopes up to 1300 meter elevation in the Himalayas.

In spite of the presence of beta- N-oxalyl-L-alpha-beta-diaminopropionic acid (ODAP a neurotoxic compound), Grass pea is considered as highly nutritive crop. Because, its protein is better quality than other pulses. Additionally the Grass pea seeds are used as complementary or source of calories (351 cal/100 gm of seeds), and endowed with about 58.2% carbohydrates, 28% protein, 0.6% fat and three gram minerals per 100 grams of seeds. However, not much research work has been conducted to screen the germplasm against resistance to powdery mildew disease.

In 1969, a disease-causing mild mottle was noticed in experimental plot at New Delhi in India. Flexuous viron 750 nm lengths were associated with the disease. Powdery mildew is caused by numbers of pathogens like *Erysiphe polygoni* D.C., *Erysiphe communis* f.sp. lathyri and *Erysiphe pisi* pv. Pisi. Among the diseases powdery mildew causes heavy losses to the crop. The reduction in photosynthesis activity and physiological changes are considerably high which leads to potential decrease in yield (20-40%) depending upon the stage and time at which the powdery mildew disease appears. Understanding the importance of disease the, severity and losses caused by powdery mildew. The present investigations were undertaken to screen Grass pea germplasm for their resistance of powdery mildew to fungicides in field as well as in vitro was tested.

**Materials and Methods**
The experiment was conducted during *rabi* season 2012 in Dept. of Botany, College of Agriculture, Nagpur, Dr. PDKV, Akola, India.

**Material fungicides used in the experiment are listed in Table 1.**

| Sr. No. | Treatments No. | Common Name          | Conc. In % | Trade Name |
|---------|----------------|----------------------|------------|------------|
| 1       | T1             | Tebuconazole         | 0.01       | Foliqur    |
| 2       | T2             | Hexaconazole         | 0.01       | Contaf     |
| 3       | T3             | Carbendanzim         | 0.1        | Bavistin   |
| 4       | T4             | Mancozeb             | 0.25       | Indofil M-45 |
| 5       | T5             | Wettable sulphur     | 0.25       | Sulfil     |
| 6       | T6             | Tridemorph           | 0.1        | Calixin    |
| 7       | T7             | Chlorothalonial      | 0.2        | Kavach     |
| 8       | T8             | Control              | -          | -          |

**Experimental layout**
The experiment was laid out in Randomized Block Design (RBD) with three replications and 8 treatments, in such a fashion that after every 15-test lines one resistance check-I namely ‘RLK-279’ and one susceptible check-II ‘BioR-231’ was sown with 3-meter row length and 30 cm apart maintaining good plant stand.
Effect of Temperature on the Germination of Powdery Mildew

Conidia of powdery mildew from young infected leaf were mixed in sterile distilled water and one drop of the spore suspension was put on cavity slide with three replications. The cavity slides were incubated in incubator (BOD) at desired temperature viz., 10°C, 15ºC, 20ºC, 25ºC, 30ºC, and 35ºC for 24 hrs. respectively. Germination count of conidia was recorded at each temperature level.

Management of Powdery Mildew of Grass pea

Efficacy of Chemical Fungicides

In vitro evaluation of fungicides, were conducted by hanging drop method using inhibition of spore germination technique.

Hanging Drop Method

Preparation of Spore Suspension

Fresh Grass pea leaves infected with powdery mildew were collected from unsprayed plants. Superficial fungal growth was scrapped with brush and incorporated in sterile distilled water.

Preparation of Moist Chamber

The Petri plates of ten cm diameter were used for preparation of moist chamber. Two moist blotter papers were kept at the bottom and one moist blotter paper kept in the top of Petri plate. Two glass rods were kept at the bottom on the moist blotter paper in Petri plate. One drop spore suspension mixed with one drop of given fungicides suspension. A loopful above suspension was taken on clean cover slip and later inverted on cavity slides. These slides were placed on the glass rod and incubated for 24 hours at room temperature.

At the end of incubation period slides were removed from Petri plates and observed under light microscope. Total number of oidia per microscopic field and number of oidia germinated were recorded and percentage inhibition was calculated by following formula.

\[
PI = \frac{C - T}{C} \times 100
\]

Where:
- \( PI \) = Per cent inhibition of germination
- \( C \) = Per cent of oidia germinated in control
- \( T \) = Per cent of oidia germinated in treatment

The experiment was laid out in Randomized Block Design (RBD) with three replications and 8 treatments (Spacing 30 x 10 cm, Seed rate; 40 kg/ha, Sowing Method; Drilling)

Preparation of Fungicidal Suspension

Each fungicidal suspension was prepared in water in plastic container (Bucket) according to concentration as it has been given in treatment details. Two sprays were given after initiation of disease by using knapsack sprayer.

### Table 2: Genotypes used in experiment

| Sr. No. | Genotypes | Sr. No. | Genotypes | Sr. No. | Genotypes | Sr. No. | Genotypes |
|---------|-----------|---------|-----------|---------|-----------|---------|-----------|
| 1       | L-01      | 16      | L-16      | 31      | L-31      | 46      | L-46      |
| 2       | L-02      | 17      | L-17      | 32      | L-32      | 47      | L-47      |
| 3       | L-03      | 18      | L-18      | 33      | L-33      | 48      | L-48      |
| 4       | L-04      | 19      | L-19      | 34      | L-34      | 49      | L-49      |
| 5       | L-05      | 20      | L-20      | 35      | L-35      | 50      | RLK-1093  |
| 6       | L-06      | 21      | L-21      | 36      | L-36      | 51      | RLK-602   |
| 7       | L-07      | 22      | L-22      | 37      | L-37      | 52      | RLK-1045  |
| 8       | L-08      | 23      | L-23      | 38      | L-38      | 53      | JRL-115   |
| 9       | L-09      | 24      | L-24      | 39      | L-39      | 54      | RLK-240   |
| 10      | L-10      | 25      | L-25      | 40      | L-40      | 55      | Ratan     |
| 11      | L-11      | 26      | L-26      | 41      | L-41      | 56      | Prateek   |
| 12      | L-12      | 27      | L-27      | 42      | L-42      | 57      | Mohateora |
| 13      | L-13      | 28      | L-28      | 43      | L-43      | 58      | BioR-208  |
| 14      | L-14      | 29      | L-29      | 44      | L-44      | 59      | BioR-222  |
| 15      | L-15      | 30      | L-30      | 45      | L-45      | 60      | JRL-16    |
Screening of genotypes

Grass pea genotype were screened against powdery mildew disease and periodical observation were recorded in 0-5 scale (IIPR, Kanpur) on disease intensity

| Grade | Description                                      | Reaction               |
|-------|---------------------------------------------------|------------------------|
| 0     | Plants free from infestation                      | Highly resistant (HR)  |
| 1     | Few plants showing traces to 10% infection on leaves, stems free from infection | Resistant (R)          |
| 2     | Slightly infection with fine coating of Powdery growth on leaves covering 10.1 to 25% leaf area, slight infection on stems, pods usually free | Moderately resistant (MR) |
| 3     | Dense Powdery coating covering 25.1 to 50% leaf area moderate infection stems, slight infection on pods | Moderately susceptible (MS) |
| 4     | Dense Powdery coating covering 50.1 to 75% leaf area, stem heavily and pods moderately infected, infected portion turn grayish | Susceptible (S)        |
| 5     | Severe infection with dense Powdery growth covering more than 75% area of the whole plant including pods, plants resulting in premature defoliation and drying | Highly susceptible (HS) |

Plant disease intensity was recorded on randomly selected 10 plants in the field. The per cent disease index was calculated by following formula

\[
PDI = \frac{\text{Summation of grades}}{\text{No. of leaves x Highest observed grade}} \times 100
\]

**Statistical Analysis**

All the data were statistically analyzed as per the procedure mentioned in statistical procedure for agricultural research.9

**Results and Discussion**

The investigation on “Powdery mildew (Erysiphe pisi) management studies in Grass pea (Lathyrus sativus L)” were carried out to find out the resistant variety against powdery mildew effect of fungicides on the disease incidence, per cent disease control and yield of Grass pea.

**In Vitro Studies of Powdery Mildew of Grass pea**

Temperature influences the disease development under natural condition. In order to know the optimum temperature requirement of powdery mildew pathogen, the conidia of *Erysiphe pisi* were exposed to different temperature under control condition.

| Levels of Temperature (°C) | Conidia Germination Percentage (after 24 hours) |
|---------------------------|-----------------------------------------------|
| 10                        | 5.14 (13.08)                                  |
| 15                        | 16.77 (24.16)                                 |
| 20                        | 19.18 (25.97)                                 |
| 25                        | 32.04 (34.46)                                 |
| 30                        | 29.69 (33.02)                                 |
| 35                        | 20.90 (27.13)                                 |

‘F’ test Sig.

SE(m) ± 0.71

CD P = 0.01 2.75

*Figures in parenthesis are sin value

Temperature range from 25-30°C was found most favorable for conidial germination which was statistically significant and at par with each other (Table 4). As the temperature increases the conidial germination was also found increases from 10°C to 25°C. However, conidial germination...
reduced gradually after 30°C (Table 4). This later on decreases gradually after 30°C. There was 12 per cent reduction in conidial germination from 25°C to 35°C within 24 hrs. This indicates that temperature influence greatly on conidial germination (Table 4).

Similar results were reported by Paulech (1969)\textsuperscript{10} which obtained optimum temperature for germination of conidia \textit{E. polygoni} at 25°C, Saharan and Sheoran (1988)\textsuperscript{11} obtained maximum germination of conidia \textit{E. cruciferum} at temperature of 21°C. Badgujar (1995)\textsuperscript{12} also found maximum germination of conidia \textit{E. polygoni} at a temperature range of 25 to 30°C.

**Effect of fungicides on conidial germination in vitro**

The data on effect of Tebuconazole, Hexaconazole, Carbendazim, Mancozeb, Wettable sulphur, Tridemorph, Chlorothalonial (fungicides) on conidial germination were presented in Table 5 below

| Sr. No. | Fungicides     | Conc.% | Conidial Germination | % Reduction over control |
|---------|----------------|--------|-----------------------|-------------------------|
| 1       | Tebuconazole   | 0.01   | 2.6                   | 94.71                   |
| 2       | Hexaconazole   | 0.01   | 1.13                  | 97.7                    |
| 3       | Carbendazim    | 0.1    | 1.4                   | 97.02                   |
| 4       | Mancozeb       | 0.25   | 2.8                   | 94.30                   |
| 5       | Wettable Sulphur| 0.25  | 5.46                  | 88.89                   |
| 6       | Tridemorph     | 0.1    | 0.80                  | 98.37                   |
| 7       | Chlorothalonial| 0.2    | 4.20                  | 91.45                   |
| 8       | Control        | -      | 49.13                 | -                       |

F- test  Sign  SE(m)  0.603  CD (0.05)  1.791

The data indicated that amongst the seven fungicides tested, Tridemorph was found to be most effective this was not significantly different from Hexaconazole and Carbendazim in reducing the per cent conidial germination of \textit{E. Pisi} (Table 5). Chlorothalonial and Wettable Sulphur showed minimum efficacy and it was significantly different from other fungicides tested except Tebuconazole and Mancozeb in respect to reduction in germination of conidia of \textit{Erysiphe pisi}.

Nawaz and Narayanswami (1983) also reported Karathane, Bavistin, Wettable Sulphur and Benlate to be most effective fungicides.\textsuperscript{13} While, Malani (1998)\textsuperscript{14} reported Hexaconazole and Tridemorph completely inhibited conidial germination at 0.1 and 0.15 % conc. respectively and these findings were in conformities with the present investigation where conidial germination was arrested at 0.1 % conc. with Tridemorph.

**Control of Powdery Mildew of Grasspea in In Vivo.**

Eight fungicides viz Tebuconazole (foliqr), Hexaconazole (contaf), Carbendazim (bavistin), Mancozeb (Indofil M-45), Wettable sulphur (sulfil), Tridemorph (calixin), Chlorothalonial (kavach) along with untreated control were tested against Grasspea powdery mildew in field conditions. The results on efficiency and yield are presented in table 6 below.

The data presented in Table 6 revealed that all the fungicides used were found to be effective in reducing the incidence and intensity of powdery mildew and enhancing the yield as compare to control. During the investigation the disease development and intensity was not observed high as compare to the previous year. This might be due to unfavorable condition particularly the rise in temperature during 47\textsuperscript{th}, 48\textsuperscript{th} and 49\textsuperscript{th} metrological week. The maximum temperature was recorded more than
30°C which affects greatly on conidial germination in the field. Hence, overall disease ratings were very low. However, the fungicides Tridemorph (0.1%) recorded minimum disease intensity (8.41%) which was followed by Hexaconazole (9.33%), Tebuconazole (9.42%), Carbendazim (9.65%) and found significantly superior and at par over the rest of treatment after 1st spray (Table 6).

### Table 6: Efficacy of fungicides against powdery mildew

| Treatments          | After 1st spray | After 2nd spray | Grain yield (Kg/ha) | Yield |
|---------------------|-----------------|-----------------|---------------------|-------|
|                     | PDI             | PDI             | PDC                 |       |
| Tebuconazole (0.01%)| 11.20 (3.35)    | 9.42 (3.06)     | 63.97               | 1043  |
| Hexaconazole (0.01%)| 10.82 (3.28)    | 9.33 (3.05)     | 64.32               | 1109  |
| Carbendanzium (0.1%)| 10.94 (3.31)    | 9.65 (3.1)      | 63.09               | 1096  |
| Mancozeb (0.25%)    | 11.64 (3.41)    | 10.58 (3.2)     | 59.54               | 931   |
| W Sulphur (0.25%)   | 13.81 (3.71)    | 12.62 (3.5)     | 51.74               | 854   |
| Tridemorph (0.1%)   | 9.76 (3.12)     | 8.41 (2.90)     | 67.83               | 1233  |
| Chlorothalonial (0.2%) | 12.72 (3.57)   | 11.53 (3.4)     | 55.90               | 886   |
| Control             | 24.33 (4.93)    | 26.15 (5.1)     | 737                 |       |

*F test*  

| SE(m)     | Sig | Sig | Sig |
|-----------|-----|-----|-----|
| 0.714     |     |     |     |
| 0.658     |     |     |     |
| 2.121     | 1.955 |     |     |

*CD(0.05)*

| 181.81 |

Figures in parenthesis are square root value

After 2nd spray more or less similar trend was noticed in reducing the infection of powdery mildew with the disease control in the range of 67.83 to 64.32% with significant superiority over the rest of treatment (Table 6).

However, the maximum yield was recorded with Tridemorph (1233 kg/ha), followed by Hexaconazole (1109 kg/ha) and Carbendazim (1096 kg/ha) which was significantly superior and at par over rest of fungicides under study (Table 6). The fungicide Tebuconazole was also found to enhance yield of grass pea significantly as compare to rest of treatments. The increased yield was 67.29% in Tridemorph to 50.47% and 48.71% in Hexaconazole and Carbendazim respectively.

Several authors also reported the effectiveness of Tridemorph and Dinocap against the powdery mildew pathogen on different crops Raut and Wangikar (1979), Nema and Krishna (1982), Singh and Singh (1982), Upadhayay and Gupta (1994) and Upadhayay and Singh (1994) also reported effectiveness of fungicides against powdery mildew. Malani *et al.* (1998) reported that Tridemorph, Hexaconazole and Carbendazim were effective in reducing the disease severity by 76.6%, 74.4% and 69.8% respectively against powdery mildew of Grass pea. Begum (1989) reported that Propiconazole was found best followed by Tridemorph, Thiavat and Dinocap against powdery mildew of field pea. Khosla *et al.* (1988) reported that Bavistin, Bayleton, Calixin, Karathane and Microsulf gave effective control of *Erysiphe polygoni* on Green Gram and Black Gram.

Among different treatment application of Tridemorph showed the highest increase yield over untreated control, it was followed by Hexaconazole, Carbendazim and Tebuconazole. Similarly, some other workers also reported the increase in yield by spraying of almost same fungicides include Singh and Singh (1982), Raut *et al.* (1986) and Malani (1998).

### Screening of Genotypes

In the present investigation 60 Grass pea genotypes with a resistance check – RLK-279’ and susceptible
check-II ‘BioR-231’ were sown on 18th October 2011. After every fifteen genotypes one resistant and one susceptible line were grown to maintain the infection of powdery mildew. Observations were recorded as scale given by IIPR, Kanpur. From the observation the per cent disease incidence (PDI) were calculated. The disease reaction was grouped in five categories, highly resistance (HR), Resistant (R), moderately resistance (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS).

The powdery mildew reactions of different Grass Pea genotypes are given in Table 7 and 8.

### Table 7: Screening of genotypes against powdery mildew

| Sr. No. | Genotypes | Disease Infection (%) | Disease reaction | Sr. No. | Genotypes | Disease Infection (%) | Disease reaction |
|---------|-----------|-----------------------|------------------|---------|-----------|-----------------------|------------------|
| 1       | L-01      | 2.22                  | R                | 31      | L-31      | 0.00                  | HR               |
| 2       | L-02      | 5.00                  | R                | 32      | L-32      | 25.10                 | MS               |
| 3       | L-03      | 1.11                  | R                | 33      | L-33      | 12.10                 | MR               |
| 4       | L-04      | 25.10                 | MS               | 34      | L-34      | 25.20                 | MS               |
| 5       | L-05      | 13.33                 | MR               | 35      | L-35      | 9.00                  | R                |
| 6       | L-06      | 1.33                  | R                | 36      | L-36      | 2.50                  | R                |
| 7       | L-07      | 25.20                 | MS               | 37      | L-37      | 8.44                  | R                |
| 8       | L-08      | 10.80                 | MR               | 38      | L-38      | 4.22                  | R                |
| 9       | L-09      | 0.00                  | HR               | 39      | L-39      | 4.91                  | R                |
| 10      | L-10      | 6.92                  | R                | 40      | L-40      | 12.08                 | MR               |
| 11      | L-11      | 11.51                 | MR               | 41      | L-41      | 1.66                  | R                |
| 12      | L-12      | 7.19                  | R                | 42      | L-42      | 26.10                 | MS               |
| 13      | L-13      | 13.18                 | MR               | 43      | L-43      | 3.75                  | R                |
| 14      | L-14      | 15.50                 | MR               | 44      | L-44      | 1.00                  | R                |
| 15      | L-15      | 0.00                  | HR               | 45      | L-45      | 1.50                  | R                |
| 16      | L-16      | 12.10                 | MR               | 46      | L-46      | 26.10                 | MS               |
| 17      | L-17      | 0.00                  | HR               | 47      | L-47      | 3.24                  | R                |
| 18      | L-18      | 25.10                 | MS               | 48      | L-48      | 16.66                 | MR               |
| 19      | L-19      | 26.10                 | MS               | 49      | L-49      | 12.50                 | MR               |
| 20      | L-20      | 16.66                 | MR               | 50      | BioR-208  | 25.30                 | MS               |
| 21      | L-21      | 1.66                  | R                | 51      | BioR-222  | 0.00                  | HR               |
| 22      | L-22      | 8.33                  | R                | 52      | RLK-1093  | 3.84                  | R                |
| 23      | L-23      | 20.10                 | MR               | 53      | RLK-602   | 0.00                  | HR               |
| 24      | L-24      | 12.96                 | MR               | 54      | RLK-1045  | 0.00                  | HR               |
| 25      | L-25      | 11.25                 | MR               | 55      | RLK-240   | 1.50                  | R                |
| 26      | L-26      | 12.50                 | MR               | 56      | JRL-16    | 0.00                  | HR               |
| 27      | L-27      | 4.16                  | R                | 57      | JRL-115   | 0.00                  | HR               |
| 28      | L-28      | 5.00                  | R                | 58      | Prateek   | 8.51                  | R                |
| 29      | L-29      | 0.00                  | HR               | 59      | Mohateora | 7.90                  | R                |
| 30      | L-30      | 18.00                 | MR               | 60      | Ratan     | 0.00                  | HR               |
| Check-I | 0.00      | HR                    | Check-II        | 26.66   | MS        |
Table 8: Reaction of genotypes against powdery mildew

| Sr. No. | Disease Infection (%) | Reaction | Total Genotypes | Genotypes |
|---------|-----------------------|----------|-----------------|-----------|
| 1       | 0.00                  | HR (free from disease) | 11             | L-09,L-15,L-17,L-29,L-31,BioR-222, RLK-602,RLK-1045,JRL-16,JRL-115, Ratan and RLK-279 |
| 2       | 0.1-10%              | R        | 24              | L-01,L-02,L-03,L-06,L-10,L-12,L-21, L-22,L-27,L-28,L-35,L-36,L-37,L-38, L-39,L-41,L-43,L-44,L-45,L-47,RLK-193, RLK-240,Prateek and Mohateora |
| 3       | 10.1-25%             | MR       | 16              | L-05,L-08,L-11,L-13,L-14,L-16,L-20,L-23, L-24,L-25,L-26,L-30,L-33,L-40,L-48,L-49 |
| 4       | 25.1-50%             | MS       | 09              | L-04,L-07,L-18,L-19,L-32,L-34,L-42,L-46, BioR-208 and BioR-231 |
| 5       | 50.1-75%             | S        | Nil             | - |
| 6       | 75.1-100%            | HS       | Nil             | - |

In the present investigation 60 Grass pea genotypes were screened for the disease reaction against powdery mildew under natural field condition. The results revealed that, 11 genotypes were found highly resistance (HR) along with resistant check RLK-279, while 24 genotypes showed resistance (R), 16 were moderately resistant (MR) and 9 were found moderately susceptible (MS) including check-II (susceptible) BioR-231 none of the genotypes has shown susceptible and highly susceptible (S) reaction to powdery mildew as the disease intensity was not high during the year of investigation. Different scientists namely Bharadwaj et al. (1987), Singh et al. (1988), Kapoor (1994) and Malani (1998) reported the disease reaction against powdery mildew disease in their screening study.

Thus, from the present investigation it is concluded that, Tridemorph (0.1%) or Hexaconazole (0.01%) had inhibited the growth of *E. pisi* significantly in *in vitro*. Spraying of Tridemorph (0.1%) significantly managed powdery mildew caused by *E. pisi* along with increased in yield of Grass pea in *in vivo*. However, this investigation needs further confirmation as these results were based on one-year experimentation.

**Ethical Standard**
The experiment conducted complies with laws

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**Conflict of Interest**
The authors declare that there is no conflict of interest regarding publication of this paper.

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