

## Effect of Strobilurin (Opera) on Growth and Yield of Soybean var. JS-335

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**ABSTRACT:** A field study was done to evaluate the effect of strobilurin: Opera (Pyraclostrobin + Epoxiconazol) on healthy plants of Soybean var. JS-335. Opera an agricultural fungicide derived from the fungal secondary metabolite- Strobilurins, is known to enhance growth and yield of cereals when applied on healthy plants. Soybean is a grain legume of considerable dietetic, industrial, medicinal and economic importance. Opera was sprayed on the leaves of soybean plants at 10<sup>th</sup> and 20<sup>th</sup> DAE (days after Emergence) at concentrations ranging from 0.05% to 0.3%. Unsprayed plants served as control. Although enhancements in growth were observed at all the concentrations of Opera used, it was maximum at lower concentrations viz. 0.1% and 0.15%. Biomass (plant height, fresh weight and dry weight) and yield (number of pods, number of seeds and 100 seed weight) of soybean were enhanced by Opera. Opera can be effectively used as a foliar spray under field conditions to enhance the growth, biomass and yield of soybean.

**Keywords:** Biomass, Growth, Opera, Soybean, Yield.

### INTRODUCTION

In response to rising input costs and narrowing profit margins, soybean [*Glycine max*] producers are continually looking for ways to increase soybean yield. One approach being promoted is the use of foliar fungicides, for both foliar pathogen control and nonfungicidal plant physiological changes in crops such as increased tolerance to abiotic stress, delayed senescence of the photosynthetic leaf area and modifications in the balance of plant growth regulators [1]. Opera is the latest fungicide from BASF Ltd. that combines two active ingredients i.e. epoxiconazole and pyraclostrobin respectively from the triazol and strobilurin family of fungicides. Strobilurins are one of the most important classes of agricultural fungicides belonging to a group of agrochemical fungicides. Strobilurin is produced by a basidiomycetes wood rotting fungus, *Strobilurus tenacellus* [2] that exert their mode of action by blocking electron transport in the mitochondrial respiratory chain in fungi. More specifically, they bind to the ubihydroquinone reduction site, the Qo-site of complex bc<sub>1</sub>, inhibiting electron transfer between cytochrome b and cytochrome c<sub>1</sub> in the respiratory chain [3, 4]. In addition to their antifungal effect, strobilurins have been reported to produce

simultaneous effects in plant physiology in grapevine under field conditions [5]. Enhancement of nitrogenase activity, leghemoglobin and the number of nodules on the roots are the additional non-fungicidal physiological effects of pyraclostrobin that contributes the enhancement of growth and yield of leguminous plants like soybean [6]. Application of fungicidal compounds belonging to the strobilurin and azole groups resulted in an increment of plant tolerance to different environmental stresses [7, 8].

Foliar application of pyraclostrobin resulted in reduction of ethylene biosynthesis in treated wheat shoots after short-term drought stress [9]. Similarly, horse chestnuts trees (*Aesculus hippocastanum*) treated with triazol: Epoxiconazole, propiconazole, penconazole or paclobutrazol were more tolerant to drought stress than untreated plants [10]. The triazoles are characterized by having a five-membered ring of two carbon atoms and three nitrogen atoms. They are curative and move systemically through the plant xylem. Triazole slows fungal growth through the inhibition of sterol biosynthesis [11-13]. Sterols are essential building blocks of fungal cell membranes and are inhibited at a single site by triazoles. Because of their curative activity against early fungal

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infections and their ability to redistribute in the crop, triazoles are highly effective and reliable [14].

Triazole compounds have both fungitoxic and plant growth regulating properties and they are considered much more effective than many other plant growth regulators, generally requiring low rates of applications [15, 16]. According to Inagaki [17] strobilurins can act on delaying root water uptake, resulting in postponement of soil dehydration, which contributes to a slight increase of grain yield in some wheat genotypes in the field under water deficit conditions. Now a day's Triazole/strobilurin combinations are very effective in raising seed yield. Triazole and strobilurin treatments associated with various morphological and physiological changes in plants including plant growth, increased chlorophyll levels, enlarged chloroplast, thicker leaf tissue, increased root to shoot ratio, delayed senescence, and enhancement in alkaloid production [18, 19].

There are many studies on strobilurins but generally those are reported on the basis of their fungicidal activity. However the effect of Opera (pyraclostrobin in combination with epoxiconazole) on morphological and physiological parameters in soybean has not been studied. Therefore the main objective of the present study was to explore the effect of foliar spray of triazole based strobilurin (Opera) application on the growth parameters, biomass and yield of soybean plants.

## MATERIAL AND METHODS

### Plant material

Field experiments under natural sunlight were conducted in School of Life Sciences, Indore (22.4°N), India. Seeds of soybean var. JS-335 were collected from Directorate of Soybean Research, Indore (M.P.), India. Seeds were inoculated with *Rhizobium japonicum* culture before sowing. The plants were daily watered as needed and weeds were controlled manually. The seeds were sown in plastic bags filled with mixture of sand, soil and manure (1:4:1) and kept under field conditions. The experiments were carried out in July 2014 to October 2014. Opera was sprayed on the soybean leaves at 10th and 20th DAE. Samples were taken at an interval of 10 days after 35th DAE for collecting the growth data, number of nodules and biomass per plant. Each experiment was done in triplicates of five plants each. Disease-free plants were used in our experiments and no disease was detected in untreated plants during our field experiments.

### Chemical details

**Opera:** Common name: (Pyraclostrobin (F500) + Epoxiconazole (Opus)). Exp. Code Numbers: Opera® (BASF). Labeled usage rate: applied at a rate of 1.5 liter formulation per ha. A formulation of Opera containing SE 133 g/l pyraclostrobin (F 500) + 50 g/l epoxiconazole was provided by BASF Inc. (Limburger Germany).

### Composition

**F500:** Methyl N-(2-([1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxy-methyl) - phenyl)N-methoxycarbamate (IUPAC); Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl] phenyl] methoxy-, methylester (CAS)

**Opus:** (2RS, 3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole

**Class:** Strobilurin (QoI) + Triazole (DMI).

**Molecular formula:** Pyraclostrobin - C<sub>19</sub>H<sub>18</sub>N<sub>3</sub>O<sub>4</sub>Cl.  
Epoxiconazole - C<sub>17</sub>H<sub>13</sub>ClFN<sub>3</sub>O.

### Foliar application

Opera (Pyraclostrobin + Epoxiconazole) applied on soybean leaves at 10 and 20 DAE (Days after Emergence) by foliar spray.

**Concentrations Used:** 0.05%, 0.1%, 0.15%, 0.2% 0.25%, and 0.3%. Unsprayed plants served as control.

### Growth analysis

Above ground parts like plant height, leaf area, shoot fresh weight, dry weight, number of nodes per plant and below ground parts like root length (length of longest root), root weight, number of nodules per plant and fresh weight of nodules were measured at an interval of 10 days till 65 DAE and leaf area was monitored in fully expanded third leaf from the top.

Area of the leaves was measured using portable laser leaf area meter CI-202 scanning planimeter (CID Inc., USA). Plant height was measured from apex to the starting part of the stem and the mean height of 15 plants was taken. Total plant fresh weight was taken after removing the plants and washing the roots thoroughly with water. Dry weight was obtained after oven-drying of the plants at 60 °C for 72 h and weighing on an analytical balance.

### Yield and its attributes

Plants were harvested at 100 DAE when the plants reached their physiological maturity (pod filling was complete). Yield was recorded in terms of number of

pods per plant, number of seeds per plant, seed weight per plant and 100 seed weight.

### Statistical analysis

All the data are presented in triplicates; five plants from each replica were taken for the recording of all parameters studied. The data are expressed as means  $\pm$  SEM.

## RESULTS

### Growth and Biomass

**Plant height:** Plant height was enhanced by all concentrations of opera compared to unsprayed control plants of soybean at all DAEs. (fig.1). Maximum enhancement of plant height was obtained at 45 DAE (42%) with 0.15% concentration of Opera and minimum was recorded at 65 DAE (20%) by 0.15% opera. Concentrations higher than 0.15% were less effective. Number of nodes were also enhanced along with plant height by foliar application of opera as compared to control plants at all DAEs (35, 45, 55, 65 DAE). The maximum enhancement was recorded at 45 DAE (27%) and minimum was recorded at 65 DAE (16%) by 0.15% opera.

**Leaf Area:** Area of fully expanded third leaf from the top was enhanced after opera application at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup>, 65<sup>th</sup>, DAE. Maximum promotion was recorded in 0.15% concentration at all DAEs (Fig. 2). Maximum enhancement of leaf area was obtained at 45 DAE (19%) and minimum was recorded at 65 DAE (7%) by 0.15% opera as compared to untreated control plants.

**Shoot fresh weight and dry weight:** Foliar application of opera was significantly enhanced Shoot fresh weight by as compared to control plants at all DAEs(35, 45, 55, 65 DAE) (Fig. 3).The maximum enhancement of shoot fresh weight was recorded at 45 DAE (39%) and minimum was recorded at 65 DAE (21%) by 0.15% opera

Shoot dry weight showed similar trend as shoot fresh weight by the application of opera as compared to the control plants at all DAEs (35,45,55,65 DAE) (Fig. 4). The maximum enhancement of shoot dry weight was recorded at 45 DAE (48%) and minimum was recorded at 65 DAE (15%) by 0.15% opera.

**Root length:** Root growth measured in terms of root length was significantly enhanced by all concentration of Opera at all DAEs (Fig. 5) but maximum enhancement of root length was recorded at 45 DAE (45%) and minimum enhancement was

recorded at 65 DAE (14%) by 0.15% opera as compared to control plants.

**Root Fresh weight and Dry Weight:** Root Fresh weight was enhanced by all concentration of Opera at all DAEs (Fig. 6). The maximum enhancement of root fresh weight was recorded at 45 DAE (38%) and minimum was recorded at 65 DAE (20%) by 0.15% opera as compared to control plants.

Root dry weight was enhanced by all concentration of Opera at all DAEs (Fig.7) but the maximum enhancement of root dry weight was recorded by at 45 DAE (41%) and minimum was at 65 DAE (33%) as compared to untreated control plants.

**Root Nodules:** Application of Opera enhanced both the number and size of the nodules formed on the roots (Fig. 8). The maximum numbers of nodules in the treated plants were found at 55 DAE and the number declined after 65 DAE. As compared to control plants significant enhancement was found at 45 DAE (45.1%) and minimum at 65 DAE (29.3%) by 0.15% opera.

Application of Opera enhanced the fresh weight of nodules formed on the roots. Here also the most effective concentration was 0.15% (Fig.9). The maximum enhancement in fresh weight of nodules in the treated plants was found at 45 DAE (41.8%) and minimum was at 65 DAE (20.2%).

### Yield and its attributes

**Number of pods per plant:** Number of pods per plant was increased in all the concentration opera as compared to control (Fig.10). Maximum enhancement was obtained in 0.15% opera and 0.1% i.e. 36% and 15% respectively. 0.05% 0.2%, 0.25% opera showed enhancement of 10%, 15% and 6% respectively whereas minimum enhancement was found in 0.3% opera (2%).

**Number of seeds and Hundred seed weight:** Maximum enhancement in number of seeds per plant was obtained in 0.15% and 0.1% opera (Fig.11) i.e. 32% and 23% respectively. 0.05% 0.2%, 0.25% opera showed enhancement of 16%, 20% and 16.8% respectively whereas minimum enhancement was found in 0.3% opera (12%).

100 seed weight has been altered after the foliar application of opera as compared to control (Fig.12). Maximum enhancement was obtained by 0.15% opera and 0.1% i.e. 33% and 23% respectively. 0.05% 0.2%, 0.25% opera showed a promotion of 4.3%, 20.8%, and 17% respectively over control. Minimum enhancement was found in 0.3% opera.

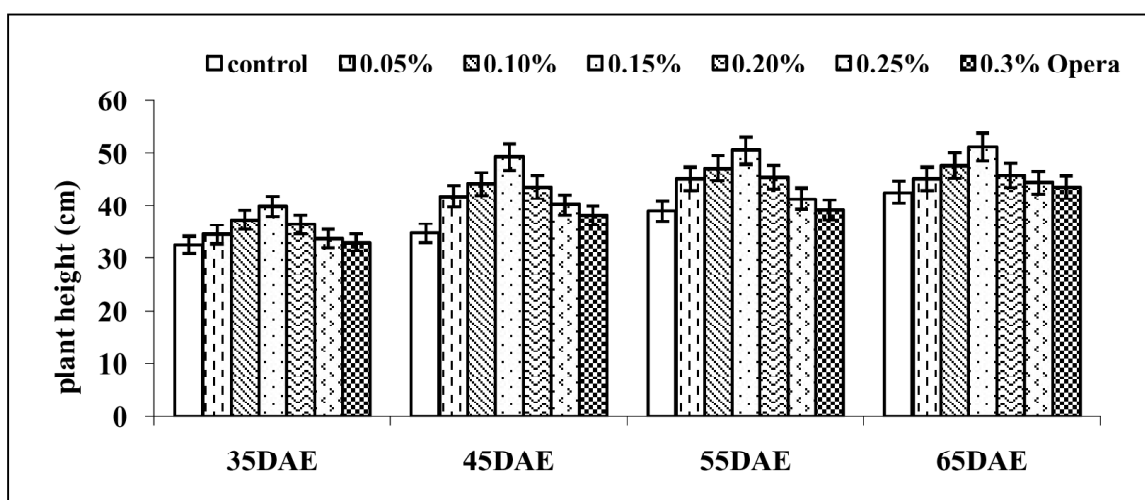


Figure 1: Effects of foliar spray of opera on plant height of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates  $\pm$  SEM for mean.

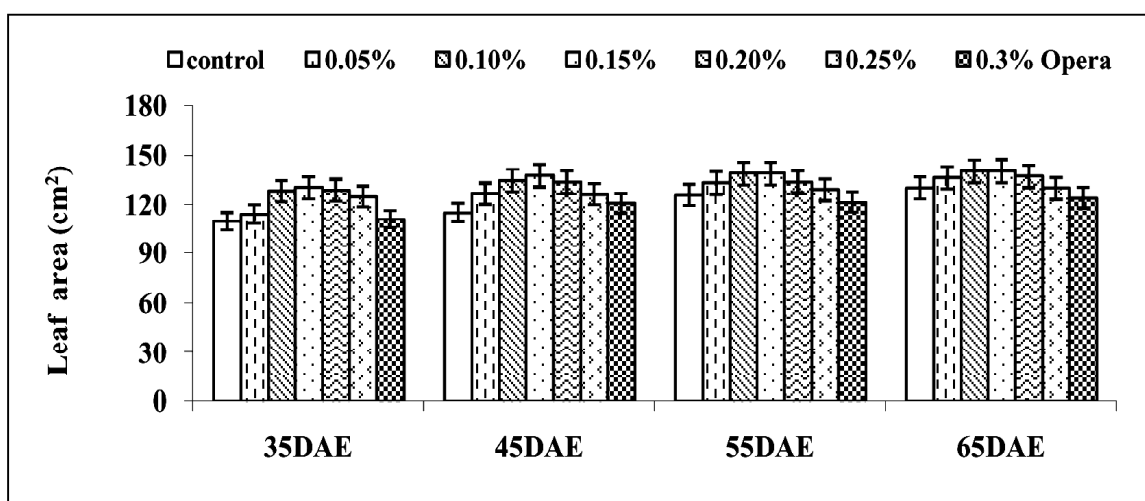


Figure 2: Effects of foliar spray of opera on leaf area of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates  $\pm$  SEM for mean.

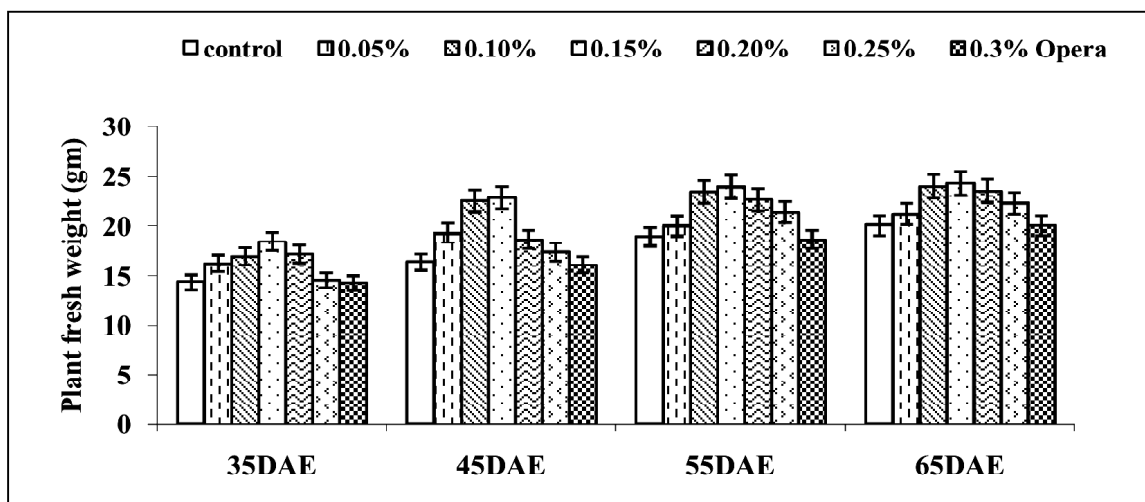


Figure 3: Effects of foliar spray of opera on Shoot fresh weight of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates  $\pm$  SEM for mean.

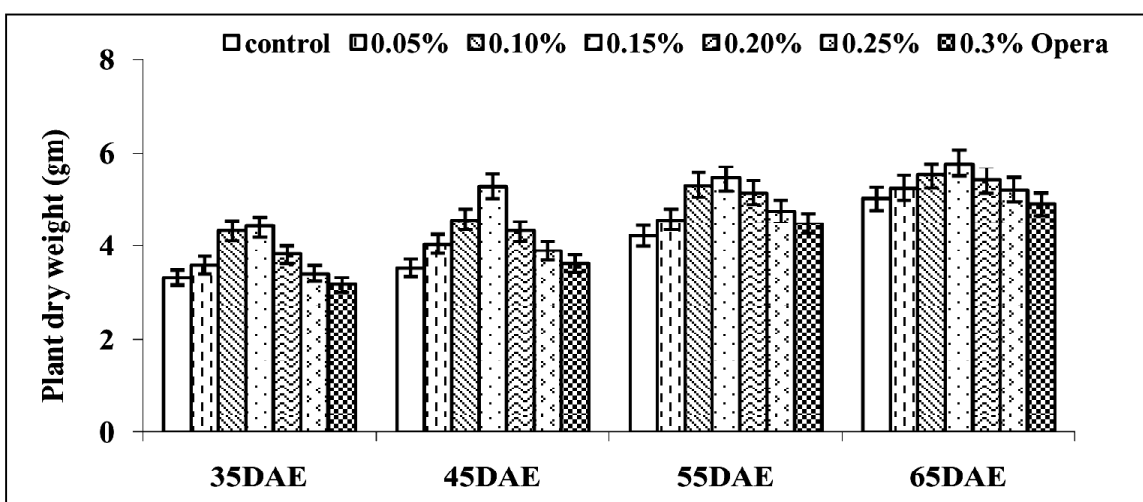


Figure 4: Effects of foliar spray of opera on shoot dry weight of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates  $\pm$  SEM for mean.

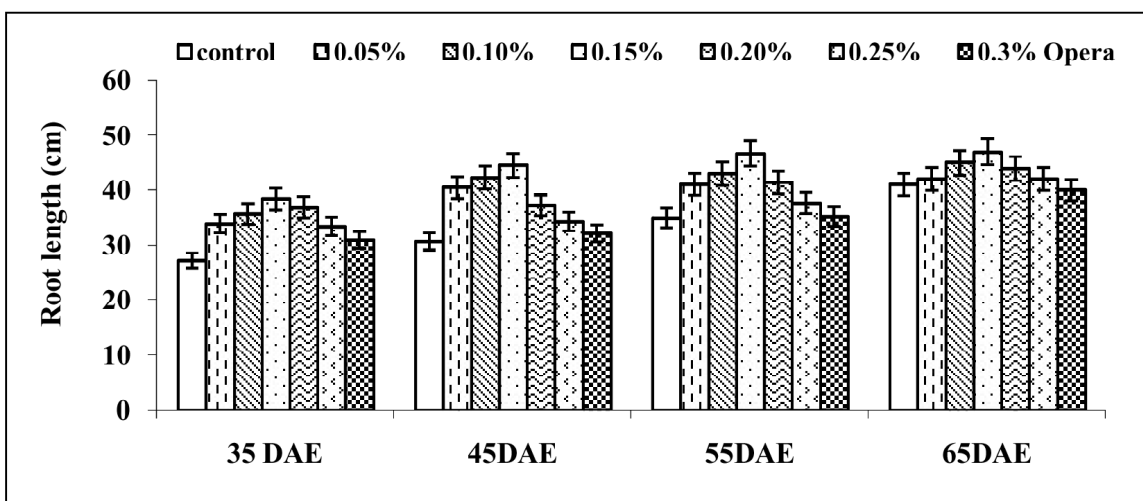


Figure 5: Effects of foliar spray of opera on root length of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates  $\pm$  SEM for mean.

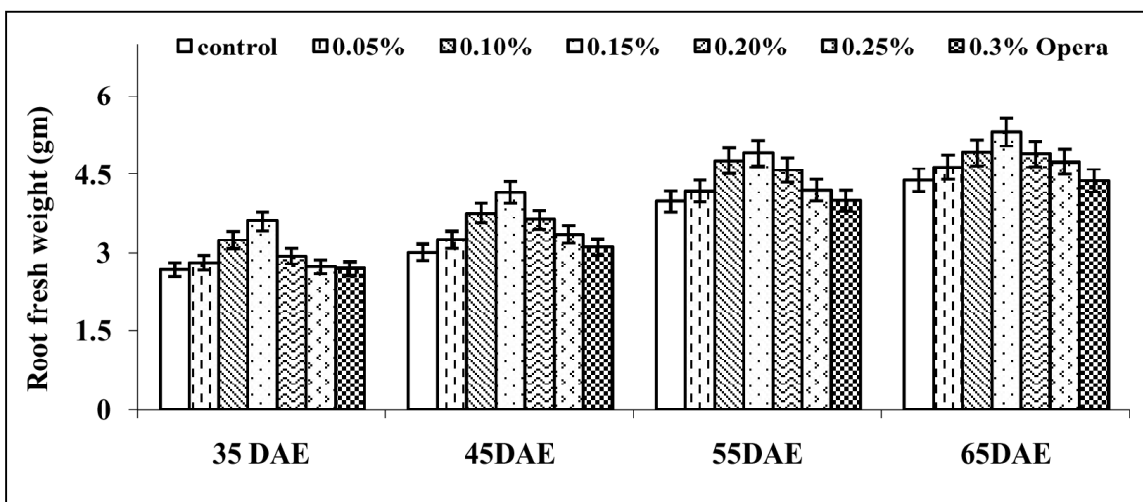


Figure 6: Effects of foliar spray of opera on root fresh weight of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates  $\pm$  SEM for mean.

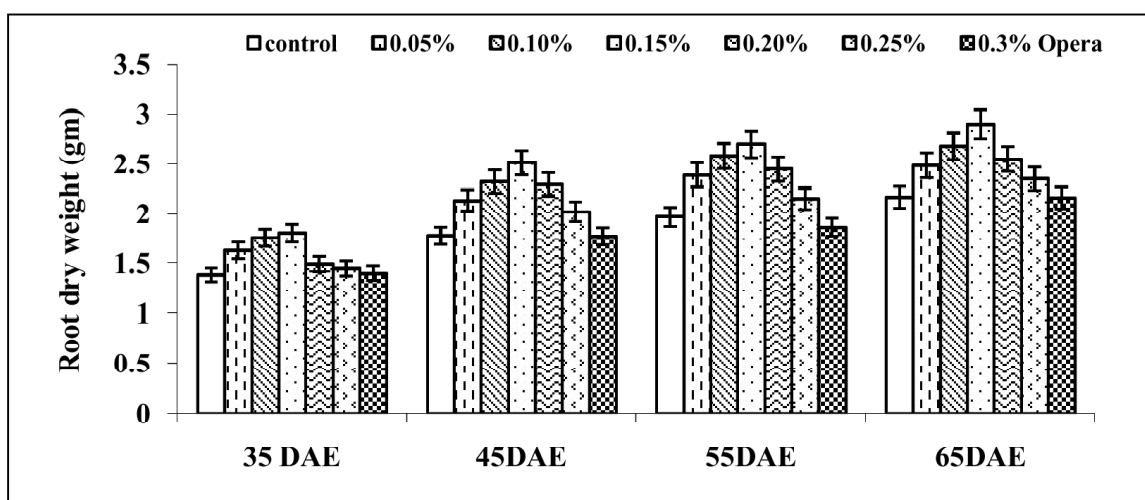


Figure 7: Effects of foliar spray of opera on root dry weight of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates ± SEM for mean.

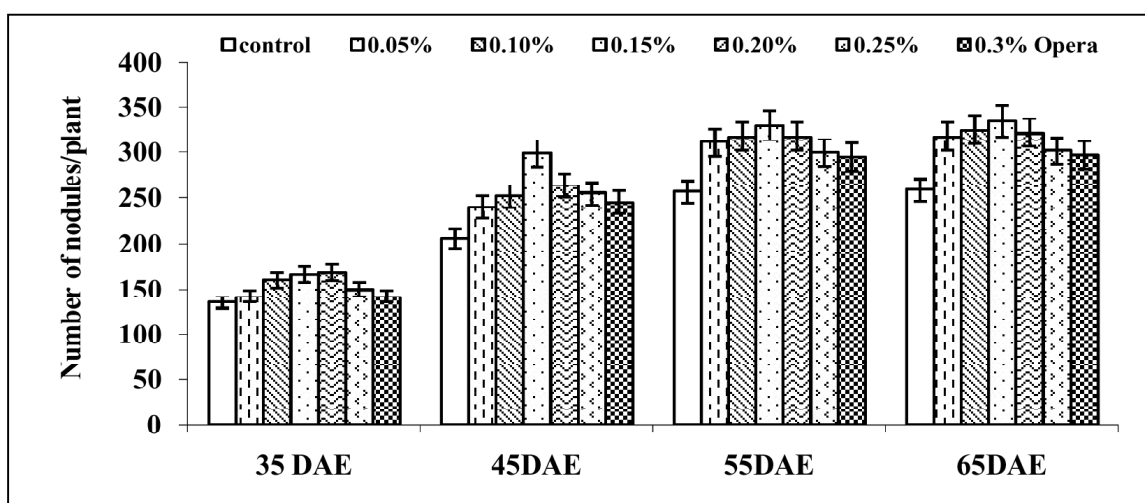


Figure 8: Effects of foliar spray of opera on number of nodules per plant of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates ± SEM for mean.

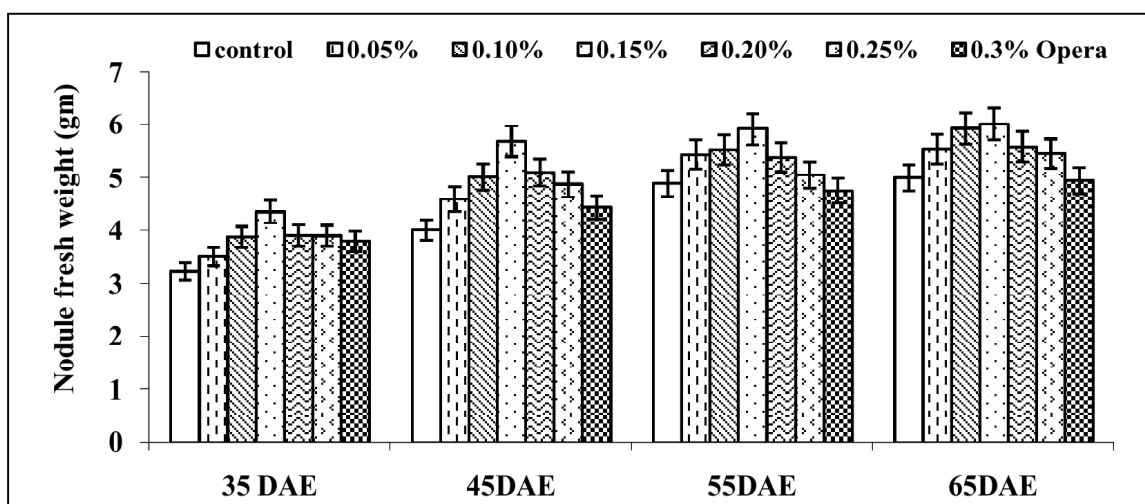


Figure 9: Effects of foliar spray of opera on nodule fresh weight per plant of soybean at different concentrations of opera at 35<sup>th</sup>, 45<sup>th</sup>, 55<sup>th</sup> and 65<sup>th</sup> DAE. The vertical bar indicates ± SEM for mean.

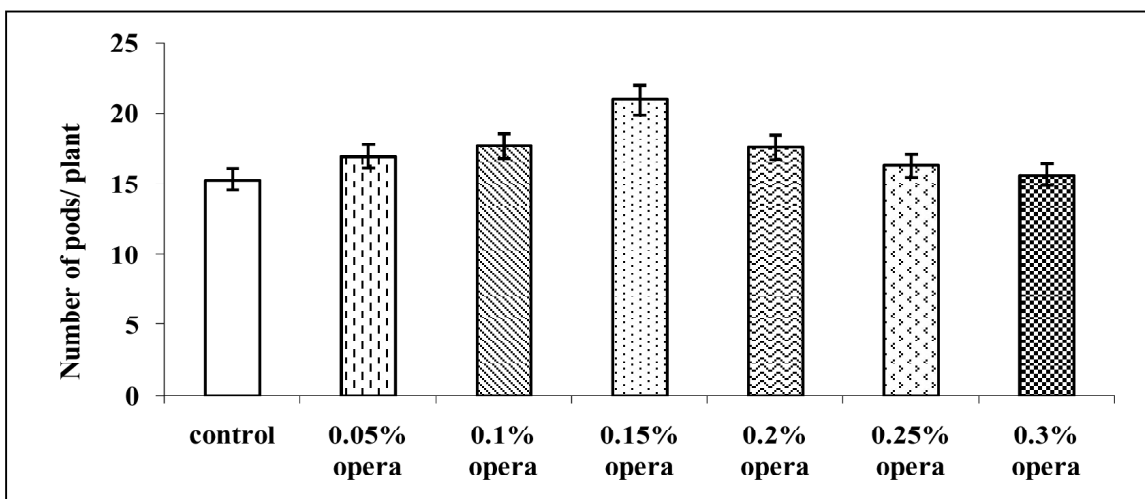


Figure 10: Effects of foliar spray of opera on number of pods per plant of soybean at different concentrations of opera. The vertical bar indicates  $\pm$  SEM for mean.

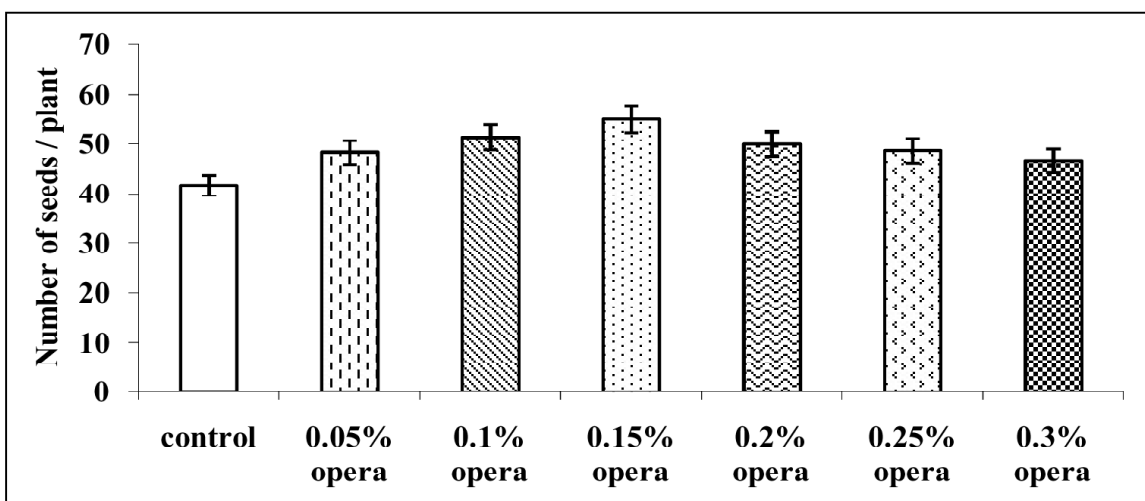


Figure 11: Effects of foliar spray of opera on number of seeds per plant of soybean at different concentrations of opera. The vertical bar indicates  $\pm$  SEM for mean.

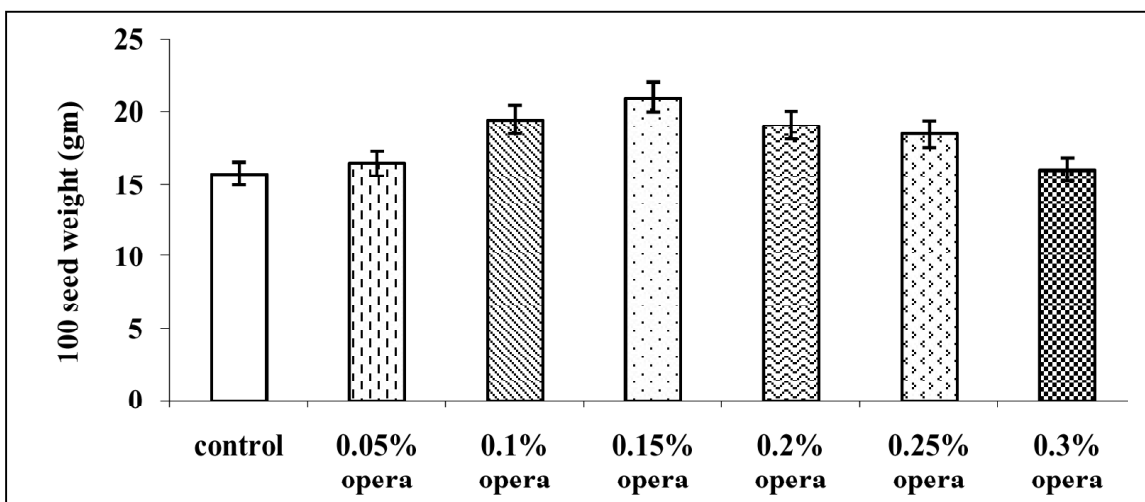


Figure 12: Effects of foliar spray of opera on 100 seed weight per plant of soybean at different concentrations of opera. The vertical bar indicates  $\pm$  SEM for mean.

## DISCUSSION

Besides fungicidal activity of opera, its foliar application enhances physiological parameters like growth, biomass and yield in soybean plants compared to untreated control plants as evident in the results. Earlier studies had shown the positive impact of pyraclostrobin on carbon and nitrogen assimilation in soybean [6, 20, 21]. Apart from nitrogenase activity pyraclostrobin enhances leghemoglobin content also which is an accessory protein that plays a crucial role in fixation of nitrogen in legumes [22]. In wheat plants strobilurins are reported to enhance the net rate of photosynthesis in the treated leaves [23, 24]. Strobilurins have shown some effects in boosting yields in wheat, corn, [25], soybean [26, 27] and in other crops. Strobilurin application also extends the life of flag leaf by retention of higher chlorophyll content [28, 29]. These observations on photosynthesis indicate a higher amount of fixation of carbon by the application of strobilurin. Improved yield is the most appreciated and profitable secondary effect and often a result of enhanced stress resistance and delayed leaf senescence characterized by reduced chlorophyll loss [30], higher protein content and increased biomass. Such effects have been previously detected with strobilurins applied in wheat and barley [7, 31].

Another reported effect of fungicides on plant physiology is an increase of the activity of antioxidant enzymes produced by strobilurins as well as by triazole application in plants exposed to water deficit. Azoxystrobin application significantly increased the activity of the antioxidative enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), protecting barley plants against injurious ozone doses [7]. In contrast to untreated plants, cowpea plants treated with propiconazole were more resistant to the adverse effects of drought stress, and additionally the activity of SOD, APX and CAT was reported to be higher in treated plants [32].

The triazole fungicide epoxiconazole is extensively used to control fungi on cereals, grapes and other crops worldwide [33, 34]. The physiological effects of strobilurin and triazole application have been studied largely using pot-grown plants grown under controlled conditions. [35]. McCartney [29] reported that the mixture of pyraclostrobin and epoxiconazole gave a significantly higher yield than epoxiconazole alone. Plots treated with epoxiconazole alone had a significantly lower level of resistance than any plot treated with an epoxiconazole/strobilurin mixture.

In the present study using strobilurin opera (F500 in a mixture with the DMI epoxiconazole) was resulted in a higher yield of soybean viz: number of pods, number of seeds, seed weight per plant and weight of hundred seeds per plant compared to untreated control. Biomass in terms of fresh weight and dry weight were significantly enhanced in opera treated plants compared to untreated plants. For the legume plants, root characteristics like root length and number of nodules plays an important role and in our findings root parameters especially number of nodules and their size were significantly enhanced as compared to control plants, this may enhance the fixation of atmospheric nitrogen. These results are particularly significant in the tropical countries like India where soybean is largely grown as a rain fed crop without using fertilizers in the field. Even if the fertilizers are used, application of opera can minimize the amount of fertilizers.

In conclusion, enhancement of plant growth parameters, the numbers of nodules on the roots are additional non-fungicidal physiological effects of opera that contributes to enhanced yield of leguminous plants like soybean. The present study provides the beneficial positive physiological effects of opera on aerial parts as well as on underground parts of soybean crop. Thus, the foliar application of the fungicide opera is very effective and can be used in increasing the seed yield of soybean under field conditions.

## ACKNOWLEDGMENTS

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