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National Academy of Agricultural Science (NAAS) Rating : 3.03

Physiological effect of Opera on growth and yield of *Trigonella foenum graecum L.*

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ABSTRACT: *Opera, an agricultural fungicide derived from the fungal secondary metabolite- strobilurin, is known to enhance growth and yield of cereals when applied on healthy plants. Trigonella is a grain legume of considerable dietetic, industrial, medicinal and economic importance. A field experiment was conducted to evaluate the effect of Opera on healthy plants of fenugreek (Trigonella foenum graecum L.) var. Pusa early. Opera was applied on the leaves of Trigonella plants at 10th and 20th Days after emergence (DAE) of seedlings at concentrations ranging from 0.05% to 0.3%. Unsprayed plants served as control. Opera treatment enhanced plant height, fresh weight; dry matter accumulation and yield parameters viz number of pods and seeds per plant, 100 seed weight. Enhancement in growth and yield parameters were observed at all the concentrations of Opera used, although the maximum enhancement was at 0.15% concentration of Opera. Our results suggest that increased biomass of treated plants showed positive correlation with increase in seed yield.*

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

INTRODUCTION

Fenugreek (*Trigonella foenum graecum L.),* an important plant in traditional medicine, is widely grown in Mediterranean countries, India and China [1]. It is commonly used as a condiment and seasoning in food preparations; is assumed to possess nutritive and restorative properties and has been used in folk medicine for centuries for a wide range of diseases including diabetes [2]. Fenugreek has important medicinal and nutraceutical properties and is also grown as a forage crop in some countries. This multi-use crop has the potential to expand into new areas, as well as increase in the area where it is traditionally grown. Therefore, its reaction to biotic and abiotic factors that can limit its production deserves special attention [3].

The seeds are used as a spice worldwide, whereas the leaves are used as green leafy vegetables in diets. Fenugreek seeds are bitter to taste and have been known over 2500 years for their medicinal qualities [4].Leaves and seeds of fenugreek contains important chemicals with lot of medicinal effects [5] like steroidal saponins; mainly diosgenin, [6, 7] which is used as raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone [8, 9]. Other bioactive constituents of fenugreek include mucilage, volatile oils, and alkaloids such as choline and trigonelline [10]. In order to optimize fenugreek yield, it is therefore necessary to improve the plant environment. Apart from soil applications, foliar spray of Opera has been shown to be a practical means to increase growth and yield of fenugreek.

Opera belongs to strobilurin class of fungicides. Strobilurin is an important class of agricultural fungicides, with a preventive mode of action and are used around the world [11]. The discovery of strobilurins was inspired by a group of natural fungicidal derivatives of beta-methoxy acrylic acid [12]. Fungal derivative strobilurin A is produced by basidiomycetes wood rotting fungi, *Strobilurus tenacellus* 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 bc1, inhibiting electron transfer between cytochrome b and cytochrome c_1 in the respiratory chain [13, 14]. Strobilurin molecules have broad spectrum fungicidal activity [15]. First strobilurin was commercially available in 1996; now many types of

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strobilurin are available like Azoxystrobin, kresoxim-methyl, metominostrobin, trifloxystrobin, picoxystrobin, pyraclostrobin, faoxadone, fenamidone. After the launching of strobilurins, and with the evolution of this group of chemical products, the concept of disease control gained new perspectives especially when considering the advantages obtained by the action of positive physiological effect on plants [16].

The combination of disease control, stress reduction and increased growth efficiency lead to the plant health benefits. In addition to their antifungal effect, strobilurins have been reported to produce simultaneous Effect in plant physiology in grapevine under field conditions [17]. Strobilurin has found to show some physiological effect like leaf greenness due to chlorophyll content [18, 19], Photosynthetic rate, water use efficiency and delayed senescence [20, 21, 11]. In United Kingdom spraying of strobilurin result in increased average yield by 0.75% in wheat and 0.34% in barle [22]. Catherine et al. [23] found increase in 10% biomass after application of pyraclostrobin, it has also been found that strobilurin has some non fungicidal physiological changes which results in increase yield in wheat *(Triticum aestivum L.)* and barley *(Hordeum vulgare)*, [21, 11, 22]. Koehle *et al.* [24] found significant increase in production of cereals after application of pyraclostrobin. McCartney [19] reported that the mixture of pyraclostrobin and epoxiconazole gave a significantly higher yield than epoxiconazole alone. The physiological effect of strobilurin and triazole application has been studied largely using pot-grown plants grown under controlled conditions. [25].

The objective of this study therefore was to assess the effect of foliar spray of Opera (Pyraclostrobin in a mixture with the DMI Epoxiconazole) on growth parameters and on yield of fenugreek var. Pusa early. Little information exists about the use of strobilurin on fenugreek, especially to document whether spraying of Opera causes nonfungicidal physiological changes and yield increases similar to what has been observed on cereals. Therefore the main objective of the present study was to explore the physiological effect of foliar application of Opera on the growth parameters, biomass and yield of Trigonella plants.

MATERIAL AND METHODS

Plant material

Field experiments under natural sunlight were conducted in School of Life Sciences, Indore (22.4°N), India. Trigonella seeds (*Trigonella foenum graecum* L.) var. Pusa early was purchased from Indian Agricultural Research Institute (IARI), Delhi, India. The seeds were inoculated with *Rhizobium japonicum* culture before sowing. The plants were daily watered as needed and weeds were controlled manually. The seeds of uniform size and shape 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 October 2014 to January 2014. Opera was sprayed on the Trigonella leaves at 10th and 20th DAE. Samples were taken at after 35 DAE (Days after emergence of seedlings) for collecting the growth data and biomass per plant. Each experiment was done in triplicate of five plants each. Disease-free plants were used and no disease was detected in untreated plants during our field experiments.

Chemical details

Opera: Common name: (Pyraclostrobin (F500) + Epoxiconezol (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)-1Hpyrazol- 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 - $C_{19}H_{18}N_3O_4Cl$. Epoxiconezol - $C_{17}H_{13}ClFN_3O$.

Foliar application

Opera (Pyraclostrobin + Epoxiconezol) applied on Trigonella leaves at 10th and 20th 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, per plant were measured at 35 DAE and area of terminal leaf was also taken at

35 DAE (Days after emergence of seedlings) 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. Total plant fresh weight was taken after removing the plants and washing the roots thoroughly with water. For dry weight determination, plant parts were dried in an oven at 60 °C for 72 h and weighing on an analytical balance. Five plants from each replica were randomly selected for recording these parameters (n = 15).

Yield components

Plants were harvested at 100 DAE when the plants reached to their physiological maturity (pod filling was complete). Yield was recorded in terms of number of pods per plant, number of seeds 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 ± SEM.

RESULTS AND DISCUSSION

The results of present study showed that foliar spray of Opera significantly enhanced some growth characteristics like plant height, leaf area, fresh weight and dry weight at all concentrations of Opera compared to unsprayed control plants of Trigonella (fig. 1) at 35 DAE.

Maximum enhancement in plant height was obtained (21%) with 0.15% concentration of Opera and minimum was recorded with 0.3% Opera. Concentrations higher than 0.15% were less effective. Area of fully expanded third leaf from the top was enhanced after Opera application at 35 DAE by foliar spray of Opera. (Fig.2). Response of Trigonella plants to foliar application of Opera in the present study is similar to that of previous studies on soybean [26].

Opera significantly enhanced shoot fresh weight and dry weight as compared to control plants at 35 DAE (Fig.3, 4). The maximum enhancement of 48% and 58% was recorded in shoot fresh weight and dry weight respectively at 35 DAE with 0.15% Opera.

Improved yield is the most appreciated and profitable secondary effect of strobilurins and often a result of enhanced stress resistance and delayed leaf senescence characterized by reduced chlorophyll loss [21], higher protein content and increased biomass. Strobilurins have shown some Effect in boosting yields in wheat, corn, [27], Soybean [28, 29] and in other crops. In the present study, yield in terms of number of pods per plant was increased in all the concentration Opera as compared to control (Fig.5). However significant enhancement was obtained in 0.15% Opera and 0.2% i.e. 46% and 42% respectively. 0.05% 0.2%, 0.25% Opera showed enhancement of 30%, 39% and 32% respectively whereas minimum of 24% was found in 0.3% Opera.

In our study maximum enhancement in number of seeds per plant was obtained in 0.15% and 0.1% Opera (Fig. 6) i.e. 42% and 33% respectively. 0.05% 0.2%, 0.25% Opera also showed enhancement of 28%, 26% and 15% respectively whereas minimum enhancement was found in 0.3% Opera (1%). Such Effects have as been previously detected with strobilurins applied in barley and wheat [30, 31].

100 seed weight has been altered after the foliar application of Opera as compared to control (Fig.7). Maximum enhancement was obtained by 0.15% Opera and 0.1% i.e. 30% and 22% respectively. 0.05% 0.2%, 0.25% Opera showed a promotion of 9%, 24%, and 13% respectively over control. There is no significant enhancement in 100 seed weight of plants treated with 0.3% Opera compared to unsprayed control plants.

According to Zhang *et al.* [32] fungicide treatments, including strobilurins, appeared to delay the senescence of wheat and increase the grain yield of wheat, owing to retarding the enhancement of active oxygen species and the decrease of antioxidative enzyme activity during aging of wheat. The results of the present study revealed that foliar application of fungicide Opera on healthy plant leaves of pusa early variety of Trigonella, improved growth and yield parameters. Our study gives new information on the response of biomass and seed yield of fenugreek to spray of Opera.

An increment of plant tolerance to different environmental stresses has resulted after the application of fungicidal compounds belonging to the strobilurin and azole groups [30, 33]. The strobilurin act by inhibiting mitochondrial respiration in fungi, disrupt the energy cycle within the fungus by halting the production of ATP, it also has activity on the plant mitochondria and reduces respiration in the plant this decrease in respiration can have a positive effect on growth. Decrease in respiration allows the plant to keep more stored carbon compounds for growth and triggers a chain reaction of positive physiological changes in the plant. Similarly triazole compounds have both fungitoxic and plant growth regulating

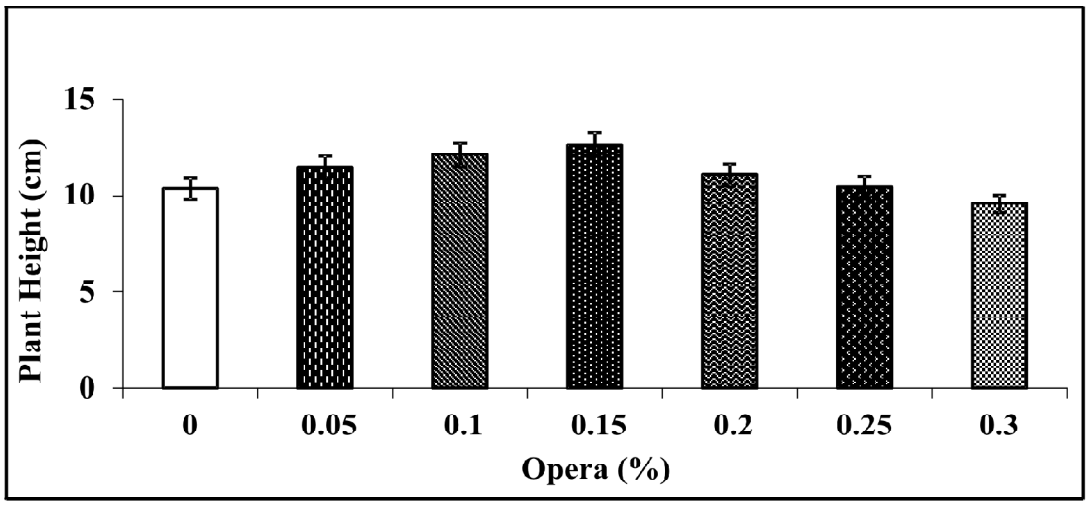

Figure 1: Effect of foliar spray of Opera on plant height of Trigonella at different concentrations of Opera at 35 DAE (Days after emergence of seedlings). The vertical bar indicates ± SEM for mean

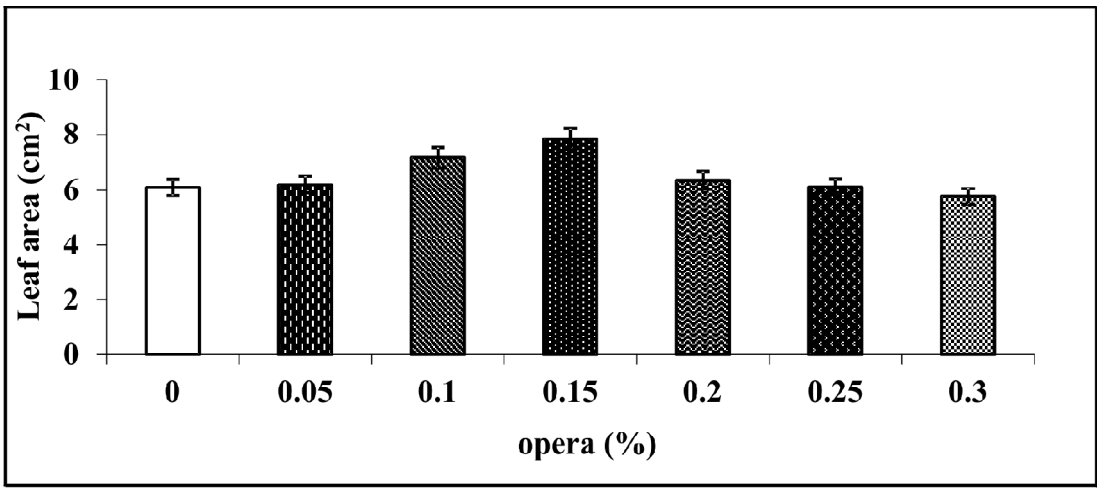

Figure 2: Effect of foliar spray of Opera on leaf area of Trigonella at different concentrations of Opera at 35 DAE (Days after emergence of seedlings). The vertical bar indicates ± SEM for mean

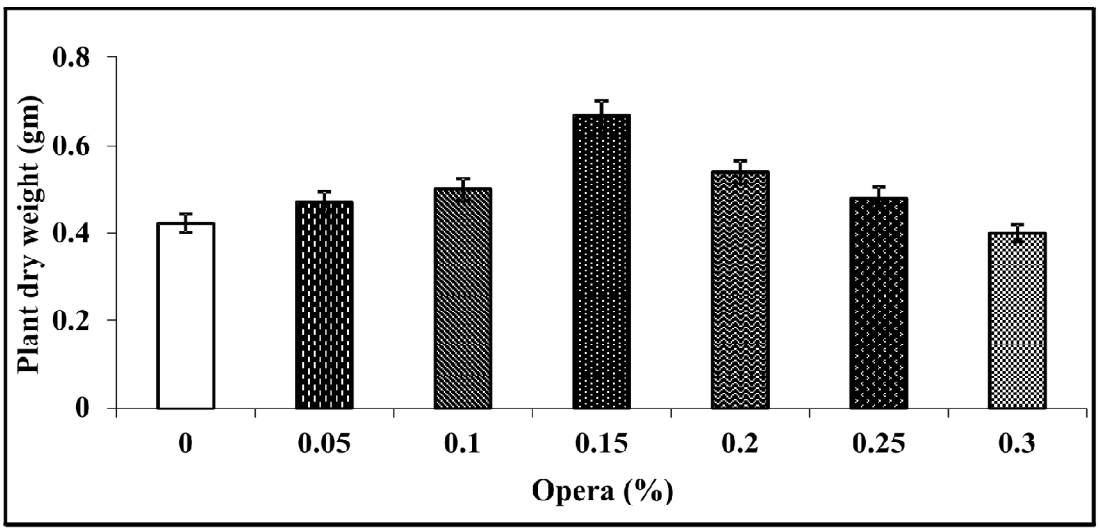

Figure 3: Effect of foliar spray of Opera on shoot fresh weight of Trigonella at different concentrations of Opera at 35 DAE (Days after emergence of seedlings). The vertical bar indicates ± SEM for mean

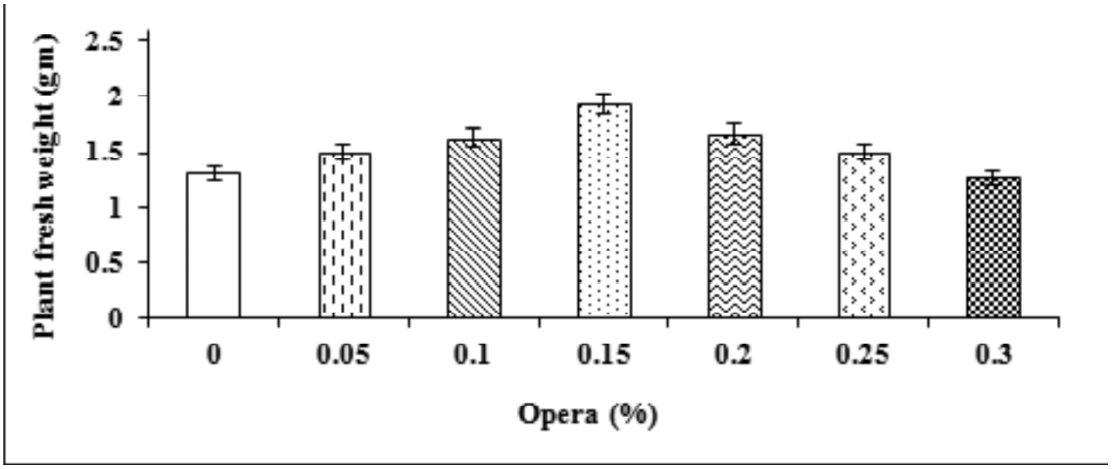

Figure 4: Effect of foliar spray of Opera on shoot dry weight of Trigonella at different concentrations of Opera at 35 DAE (Days after emergence of seedlings). The vertical bar indicates ± SEM for mean

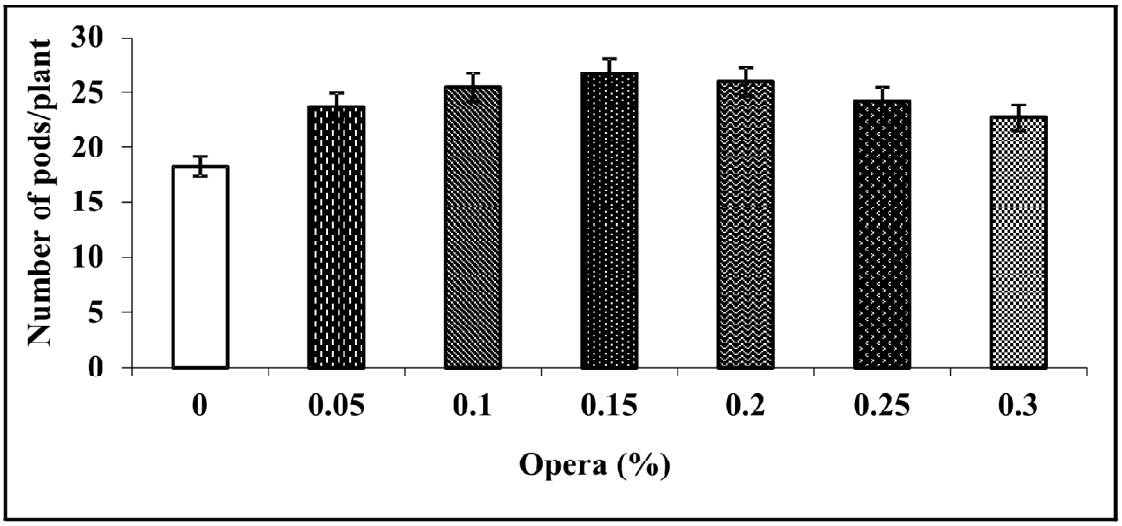

Figure 5: Effect of foliar spray of Opera on number of pods per plant of Trigonella at different concentrations of Opera. The vertical bar indicates ± SEM for mean

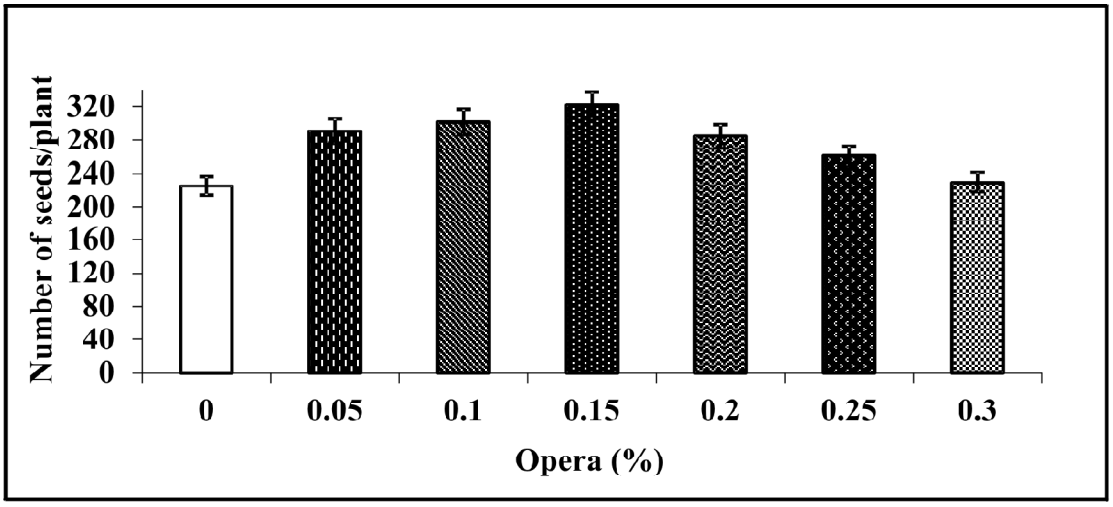

Figure 6: Effect of foliar spray of Opera on number of seeds per plant of Trigonella at different concentrations of Opera. The vertical bar indicates ± SEM for mean

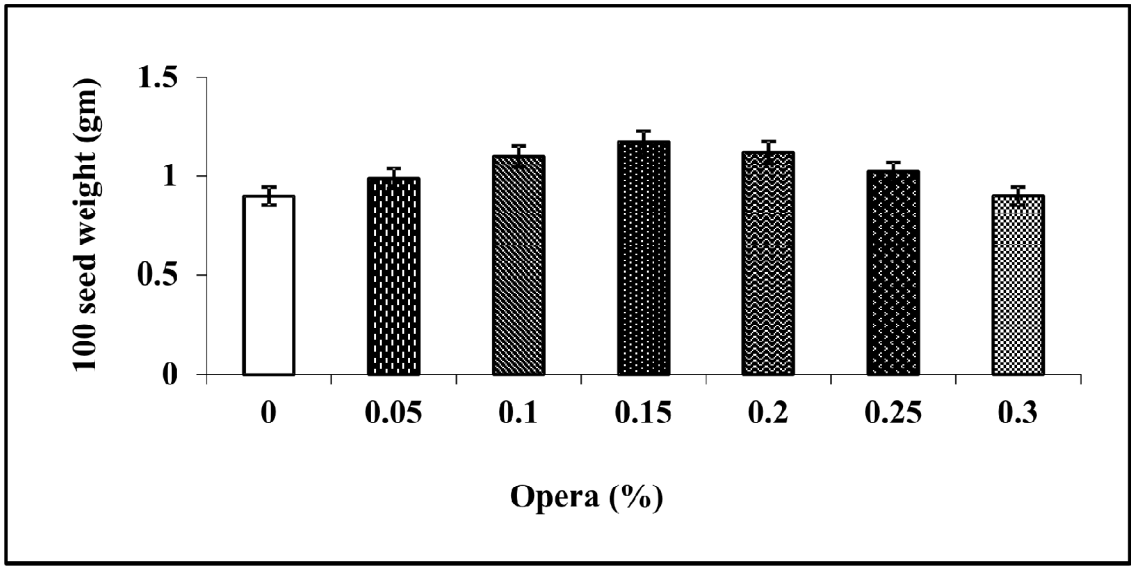

Figure 7: Effect of foliar spray of Opera on 100 seed weight per plant of Trigonella at different concentrations of Opera. The vertical bar indicates ± SEM for mean

properties and they are considered much more effective than many other plant growth regulators, generally requiring low rates of applications [34, 35]. Study using strobilurin Opera (Pyraclostrobin in a mixture with the DMI Epoxiconazole) resulted in a higher yield of soybean reported by Kanungo et al. [26].

In conclusion, our results indicated that among all the concentration of Opera (0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%), large enhancement in growth and yield was recorded with 0.15% Opera treated plants compared to untreated plants, thus suggesting that foliar application of Opera even in its low concentration is beneficial for plant growth, development and yield on commercial level.

ACKNOWLEDGMENTS

Financial support by BASF India Ltd. to Mansi Kanungo is thankfully acknowledged.

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