

## **Impact Of Agnihotra On Antibiotic Properties Of Piper Nigrum**

P.K. Mishra

(Deptt. Of Botany, Vinoba Bhave University, Hazaribag)

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**Abstract :-** Piper nigrum L. is an important medicinal plant having wide applications in Ayurveda as antibiotic. Experimentally, extract of Piper nigrum extract has proved effective on both gram -ve as well as gram +ve bacteria. Agnihotra therapy is another noteworthy systems of treatment of various diseases where fume of some select plant twig clarified butter and pinch of rice, effectively control growth of disease causing bacteria. In present study combined impact of plant extract and Agnihotra on growth of some pathogenic bacteria was studied. It was proved that Agnihotra on growth of some pathogenic bacteria was studied. It was proved that Agnihotra conclusively help in controlling growth of pathogenic bacteria. Hence Agnihotra treatment along with extract of Piper nigrum is suggested.

**Key words:-** *Agnihotra, Antibacterial activity, Piper nigrum.*

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### **I. INTRODUCTION**

Man has been confronting with disease causing microbes since time immemorial and plants have proved to be a reliable tool for fighting these disease causing pathogens. Going down the lane of history of disease management, Ayurveda, the time tested science of therapy is full of references of antibiotic properties of plants. During last few decades, synthetic antibiotics got popularity in medical science but very soon side effect of these synthetic drugs were experienced and once again whole world is returning towards herbal medicine and Ayurveda (Kumar et.al. 2013, Dandpat et.al. 2014). *Piper nigrum* L., commonly known as black pepper is one plant which has wide application in treatment of various types of diseases like, indigestion, fever, sinusitis, asthma, congestion etc Ravindran (2000). In Ayurvedic literature there is description of another system of therapy and it is known as Agnihotra Chikitsa. Agnihotra is a process during which pyre is lit in a pyramid shaped pot with the help of cow dung, twig of some plants and clarified butter. Pinch of rice is offered in flame during sunrise and sunset. The fume thus generated has been proved scientifically to possess antibacterial properties. The ash has also great potential of killing pathogen. Pathade and Abhang (2014), Mondkar (1987), Rao and Tewari (1987), Atul et.al. (2009) and Purandre and Prasad (2012) have reported efficacy of Agnihotra and ash thus generated in controlling growth of bacteria. Present experiment was conceived with aim of studying combined effect of extract of *Piper nigrum* and Agnihotra of growth of some bacteria. Multiple therapy and pluralism is being advocated in control of disease and this study is following the same direction.

### **II. MATERIAL AND METHODS**

The *Piper nigrum* seeds were ground to a fine powder before extraction. The pepper powder (50 gm) was then extracted with methanol using soxlet apparatus by continuous heat extraction for 24 hours. The extracts obtained were concentrated to dryness by evaporating the solvent under reduced pressure. The concentration thus obtained was dissolved in DMSO in such a way that the final concentration of the extract would be 1g/ml of DMSO.

#### **2.1 Disc diffusion method**

The *in vitro* antibacterial activity of the methanol extracts of pepper was carried out by disc diffusion method. Actively growing log phase cultures were mixed in soft agar (Nutrient broth with 1% agar) and plated. The extract (5µl or 5mg) was loaded onto different filter paper discs prepared from Whatman's No: 1 filter paper. The discs were then placed on the agar medium containing the cultures and incubated for 24 hrs at 37°C. The diameter of zone of growth was recorded.

#### **2.2 Minimum inhibitory concentration (MIC)**

MIC was determined by serial dilution method. Two fold serial dilution of the test compound was carried out in the nutrient broth. To each test tube 10<sup>5</sup> CFU/ml of actively growing bacterial cultures in log phase

was inoculated. The culture tubes were incubated at 37°C for 24 hours. After the incubation the tubes are checked for the growth of bacteria and MIC of that extract was determined and expressed in ppm.

### 2.3 Agnihotra experiment

A pyramid shaped copper pot, 14.5cmx14.5cm at top and 5.50cmx5.50 cm at the bottom and 6.50 cm in height was used for this experiment. Dry twigs of *Ficus benghalensis*, *Ficus glomerulata*, *Ficus religiosa*, *Butea monosperma* and *Aegel marmelos* and cow dung was lit with clarified butter of cow and pinches of rice was offered at sunrise and sunset for three minutes for seven days. Two parallel sets of experiments were set. In one set only aqueous extract of *Piper nigrum* was used whereas in another set same concentration of *Piper nigrum* extract was used along with Agnihotra practice. Zone of inhibition and minimum inhibition concentration was observed in both the conditions. Results obtained were statistically tested using Students t test.

### III. RESULT AND DISCUSSION :

Results obtained are presented in Table-1 and 2. ZOI for *Staphylococcus aureus* was recorded 22 mm in test plant extract whereas it increased to 24 when plant extract and Agnihotra treatment was used in combination. *Bacillus cereus* colony reduced to 14 mm when treated with plant only whereas Agnihotra and plant extract in combination inhibited colony to 26 mm. Reduction in colony size was reported 10 mm in case of *Streptococcus faecalis* which became double, 20 mm in combination condition. *Escherichia coli* colony was inhibited to 18mm when treated with plant extract alone whereas when plant extract treatment was combined with Agnihotra, zone reduced to 26 mm. In case of *Salmonella typhi* treatment with test plant extract minimised to 10 mm whereas combined with Agnihotra, zone reduced to 18 mm. Minimum inhibition concentration (MIC) also exhibited positive result of Agnihotra on antibacterial property of *Piper nigrum*. *Staphylococcus aureus* size reduced at 125 ppm concentration of extract of test plant but when exposed to Agnihotra inhibition was recorded at 80 ppm. Similarly inhibition of colony of *Bacillus cereus* with test plant extract and combination condition was 250 ppm and 140 ppm respectively. Extract of test plant was effective on *Streptococcus faecalis* at a concentration of 140 ppm but when Agnihotra was practised with plant extract treatment, later was effective at 90 ppm concentration. *Escherichia coli* colony exhibited reduction at 180 ppm of plant extract but Agnihotra further reduced the concentration to 100 ppm. Effective concentration of plant extract on *Salmonella typhi* was 90 ppm but plant extract worked significantly at 60 ppm even. Results clearly indicated that Agnihotra practices significantly enhance antibacterial efficacy of *Piper nigrum* extract. There is however variation so far impact of this practice on different bacteria are concerned. *Bacillus cereus* and *Streptococcus faecalis* proved to be most affected by Agnihotra as highest increase in ZOI was recorded in these two strains. Effect on other bacteria tested was also significant. So far MIC is concerned, *Bacillus cereus* and *Escherichia coli* was found more vulnerable. Change in MIC of other strains was also significant. Ulrich Berk (2016) of German Association of Homa Therapy has extensively revisited the science of Agnihotra which is also known as Homa and has conducted several experiments which conclusively establish importance of this system for treatment of various diseases. Mishra (2016) has explained chemistry of Agnihotra fume and tried to explain the mechanism behind its action. Modern researches have clearly proved efficacy of agnihotra in purifying air. The materials of agnihotra are basically cellulose and lignocelluloses. After combustion, the hydrogen atom liberated combine with O<sub>2</sub> to form water vapour. This vapour also contains compounds like thymole, engomal, pinen, terpenol, etc. and travels to great distance in all directions. Various photochemical reactions follow and oxidation-reduction, decomposition of harmful gases leads to formation of harmless components. Carbon dioxide, one of the most common toxic gases is converted into formaldehyde and the air is relatively clean after agnihotra. Formaldehyde thus formed has got antiseptic properties hence it destroy pathogenic germ present in the air.

**Table 1. Zone of inhibition (ZOI) in mm of *Piper nigrum* L. extract and extract in combination with Agnihotra on some pathogenic bacteria**

Bacteria	Zone of Inhibition(mm)	
	Methanol extract (5µl) (M ± SD, n =6)	Methanol extract + Agnihotra (5µl) (M ± SD, n =6)
<i>Staphylococcus aureus</i>	22 ± 0.8	24 ± 0.9
<i>Bacillus cereus</i>	14 ± 25	26 ± 0.8
<i>Streptococcus faecalis</i>	10 ± 0.5	20 ± 0.7
<i>Escherichia coli</i>	18 ± 0.6	26 ± 0.8
<i>Salmonella typhi</i>	10 ± 0.5	18 ± 0.5

Level of significance:  $p < 0.001$

**Table 2. Minimum inhibition concentration (MIC) in *Piper nigrum* L. extract and extract in combination with Agnihotra on some pathogenic bacteria**

Bacteria	MIC in ppm	
	Methanol extract(M ± SD, n =6)	Methanol extract + Agnihotra(M ± SD, n =6)
<i>Staphylococcus aureus</i>	125 ± 15	80 ± 10
<i>Bacillus cereus</i>	250 ± 25	140 ± 15
<i>Streptococcus faecalis</i>	140 ± 20	90 ± 10
<i>Escherichia coli</i>	180 ± 10	100 ± 15
<i>Salmonella typhi</i>	90 ± 10	60 ± 5

Level of significance:  $p < 0.005$

#### IV. CONCLUSION

On the basis of results obtained and earlier reports, Agnihotra Therapy or Homa Therapy is established as scientific and safe way of treating various types of ailments especially bacterial diseases. The technique also helps in reducing dose of medicine working probably as bioenhancer. Apart from increasing efficacy of plant extracts, it also help in conservation of medicinal plants. There is need to strengthen this neglected area of Ayurveda by conducting and supporting research in this area. This will provide a comparatively safe option for people.

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