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Effect of Foliar Sprays with Nutrients and Growth Regulators on Seeding Vigour and Enzyme Activity in Mung bean

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ABSTRACT: The present study was undertaken tofind out the response of nutrient and growth regulators on seed germination, seedling vigour and enzyme activity of mung bean (*Vigna radiata*). Treatments consisted of three varieties *viz.*; Pant Mung 2 (small seeded), Pant Mung 4 (medium seeded) and Pant Mung 5 (bold seeded) and six foliar sprays; urea 2%, NPK 2%, DAP 2%, salicylic acid 100 ppm and indole butyric acid 100 ppm and control. Foliar spray of nutrients and growth regulators was done twice 40 and 55 days after sowing. The experiment was laid out in Randomized Block Design with three replications. After harvesting, seeds were used for conductinggermination test, seedling vigour and enzyme activities. Results revealed thatgermination percentage, seedling length,seedling vigour indices, protein content, *á*- amylase and protease enzymatic activity was recorded highest in bold seeded variety Pant Mung 5. Seeding vigour of mung bean was also influenced by foliar sprays of nutrients and growth regulators. Protein content in seeds increased significantly with 2% foliar spray of urea whereas, other quality parameters such as germination percentage, seedling length, seedling dry weight, seedling vigour index -I , seedling vigour index -II,*á*- amylase and protease enzyme activity were significantly higher in foliar spray of 100 ppm salicylic acid.

Keywords: Green gram, Foliar spray, Nutrient, Growth regulator, Seed quality, Enzymatic activity

Green gram (*Vigna radiata* L. Wilczek), also known as mung bean is an important pulse crop and serves as a major source of dietary protein. In India, mung bean ranks third after bengal gram and red gram. It is a major crop of both *kharif* and summer season but maximum area is occupied in *kharif* season. Mung bean covers an area of 4.3 million hectare with a production of 2.07 million tones and an average productivity of 481 kg/ha [1]**.** In boosting agriculture production, quality of planting material plays an important role as yield per unit area of a crop mainly depends on the initial quality of the seeds, agronomic practices and use of modern inputs. Quality seed accounts for 25-30% of the total yield increase [2]**.** Seed quality is a complex phenomenon which is mainly influenced by genetic, agronomic and environmental factors. Among the agronomic factors selection of the variety, its establishment, growth and development are to be taken care of through management practices.

The improved genetic materials are highly responsive to nutrients. An adequate and timely application of nutrients helps in healthy growth, higher yield and good seed quality. Application of nutrients through foliar spray along with soil application has several advantages in supplementing the nutrition requirement of crops. Foliar nutrition has been designed to eliminate the problems like fixation and immobilization of nutrients. Hence, foliar nutrition has been recognized as an important method of fertilization in modern agriculture.

Application of nutrients through foliar spray along with soil application has several advantages in supplementing the nutrition requirement of crops. Foliar nutrition has been designed to eliminate the problems like fixation and immobilization of nutrients. Hence, foliar nutrition has been recognized as an important method of fertilization for improving the productivity and quality of seed/grains crops, if applied during flowering or pod formation stages [3]**.** Among all the nutrients, role of nitrogen is important because it is the building substances from which the protoplasm of every cell is made. Similarly, phosphorus helps in root development, crop maturation, formation and translocation of carbohydrates, and also resists the incidence of pathogen to diseases. The fertilizer with different ratio of N, P and K are highly water soluble and amenable for foliar nutrition. Pulses itself considered as a nitrogen rich source because of high nitrogen fixation capacity but their demand for nitrogen is more critical at flowering and seed development stage. Therefore, availability of nitrogen especially during reproductive stage is an important factor influencing the yield and quality of seed.

Foliar spray of growth regulators also has positive effect on plant metabolism and thus promotes yield and seed quality. Plant growth regulators (PGR's) are well known to improve physiological and biochemical processes during biotic and abiotic stresses, which directly or indirectly affect the crop yield**.** They enhance the source sink relationship and stimulate the translocation of photo assimilates, thereby increase the productivity. Foliar spray of plant growth regulators stimulates synchronized bloom, reduces flower drop, improves seed setting and ultimately increase the yield [4]. Salicylic acid (SA) is a secondary metabolite acting as analogue to growth regulating substances and helps in protein synthesis. Also, IBA promotes growth and consequently increases translocation and accumulation of building metabolites to seeds.Therefore, applying additional nutrients during flowering and post flowering period may be helpful in improving yield and quality of seeds. However, there is relatively little information available in mung bean about foliar spray in combination to soil application of nutrients on seed germination, seedling vigour and enzyme activities of mung bean crop.

MATERIALS AND METHODS

The seed material of three varieties *i.e.*, Pant Mung 2 (small seeded), Pant Mung 4 (medium seeded) and Pant Mung 5 (bold seeded) of mung bean were obtained from Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology,Pantnagar, Uttarakhand, India. The varieties were selected on their thousand seed weightbasis. The total treatments consisted of three varieties and six foliar sprays of nutrients and growth regulators *i.e.*urea 2%, NPK 2%, DAP 2%, salicylic acid 100 ppm and indole butyric acid 100 ppm and control). The crop was planted in *kharif* season 2017 with a spacing of 30cmx10cm for all the treatments. All the cultural operations were followed as per recommended cultural practices.Spraying of solutions was done as per treatment concentrations uniformly in each plot twice at 40 and 55 days after sowing. After threshing and winnowing, random seed samples of 100 seeds were taken from net harvested plot of each treatment in three replications. Their weight was weighed and multiplied with ten to calculate 1000 seed weight in grams.

For laboratory studies, the seeds harvested from each plot were considered as a separated seed lot and sampling for laboratory observations was done by hand method to obtain uniform and representative sample for following laboratory observations.

Germination (%)

Three replicates of 100 seeds were placed in between the rolled tower paper and kept in a germinator maintained at 25±2 0C and 95±2 % RH. The seedlings were evaluated after eight days according to the standard procedure for germination as described in International Rules for Seed Testing [5] and expressed in percentage.

Shoot and Root Length

At the end of germination test, ten seedlings were randomly selected for recording shoot and root length of the seedling. After removing cotyledon, the root and shoot of the seedlings were separated. Thereafter, the length of root and shoot of the individual seedlings was measured separately in cm and mean value was expressed in cm. Shoot and root length was then added to obtain seedling length and mean value was reported in centimeter per seedling.

Seedling Dry Weight

After measuring the seedling length, the samples were dried in an oven maintained at 70±2°C till the weight become constant. After proper drying, seedling dry weight was recorded and expressed as milligram per seedling.

Seedling Vigour Index

Seedling vigour index was computed by the method [6] given below.

Seedling Vigour Index I (SVI- I) = Germination (%) x Seedling length (cm/seedling)

Seedling Vigour Index II (SVI-II) = Germination (%) x Seedling dry weight (mg/seedling)

Germination Index (GI)

After conducting germination test, daily observations were recorded to count number of seeds germinated per day. On the 8^{th} day of germination,speed of germination *i.e.* Germination Index (GI) was determined by the following formula as suggested by [7].

$$\text{Germination Index} = \Sigma \frac{n}{di}$$

Where, n = number of seeds germinated on i th day.

$di = i$ th days after incubation

To work out protein per cent of seeds, nitrogen content of seed was estimated by Micro Kjeldahl method and then it was multiplied by [8]. The *α* amylase and protease enzyme activity were calculated by the method suggested by [9]. The enzyme *α* amylase was expressed as mg of starch hydrolysed/g of seed and protease was expressed as mg of tyrosine/g of seeds.

RESULTS AND DISCUSSION

Seed Germination and Seedling Vigour Parameters

The data in table 1 showed significant effect of varieties and foliar spray of nutrients and growth regulators on 1000 seed weight. The highest 1000 seed weight was noticed with bold seeded variety Pant Mung 5 (47.0 g) followed by medium seeded variety Pant Mung 4 (32.4 g) and the lowest with small seeded variety Pant Mung 2 (28.1 g). All the varieties differed significantly with each other. Thousands seed weight is an index of boldness of grains resulting from the transfer of photosynthates from source to sink [10] reported that hundred seed weight was highest in large seeded seeds over small sized seeds in pigeon pea. Similar findings were also reported in field pea [11].

Among foliar spray treatments, the boldest seeds were found with spraying of 2% DAP (36.1 g) which was significantly superior to the rest of the foliar treatments. It was followed by 2% urea spray (35.4 g). Foliar spray of urea was significantly superior to 100 ppm salicylic (34.3 g) acid and 2% NPK (34.1 g) which was at par with each other. The smallest seeds were recorded with control (32.6 g) and were *at par* with 100 ppm indole butyric acid (32.7 g). Foliar spray of nutrients decreased the interplant competition which resulted in bolder seeds. The results are in agreement with the study done on black gram [12].

Seed vigour in terms of rapidity of germination was assessed by speed of germination *i.e.* germination index. In the present study the effect of seed size on germination

Table 1. Seed index, vigour parameters and á- amylase enzyme activity of mung bean in response to different varieties and foliar spray of nutrients and growth regulators

Treatments	1000 seed weight (g)	Germination (%)	Germination Index (seedlings/ per day)	Seedling length (cm/ seedling)	SVI-I	Seedling dry weight (mg /seedling)	SVI-II	α- amylase (mg of starch hydrolyzed /g seed)
Variety								
Pant Mung 2	28.1	75.1	18.4	10.6	833	0.131	9.9	9.3
Pant Mung 4	35.4	76.6	17.3	13.1	986	0.146	11.3	9.7
Pant Mung 5	42.1	81.3	1.02	14.0	1113	0.226	18.0	11.7
SEm(±)	0.2	0.8	0.4	0.2	28	0.006	04	0.3
CD (p=0.05)	0.5	2.5	0.12	0.7	80	0.018	1.3	0.9
Foliar spray								
Control	32.6	74.4	12.3	9.3	679	0.141	10.5	9.6
Urea 2%	35.4	80.2	14.4	12.6	989	0.157	12.6	10.2
NPK 2% (12:32:16)	34.1	76.8	16.3	1.0.0	782	0.158	12.2	11.5
DAP 2%	36.1	78.8	13.1	12.7	955	0.175	13.6	10.3
SA* 100 ppm	34.3	82.8	17.0	16.4	1309	0.198	15.9	10.2
IBA **100 ppm	32.7	75.5	18.3	14.3	1146	0.176	14.2	9.6
SEm(±)	0.2	1.5	0.06	0.4	38	0.009	0.6	0.4
CD (p=0.05)	0.7	4.4	0.17	1.1	110	0.025	1.9	NS

*Salicylic acid; ** Indole butyric acid

Note: Germination test was performed two months after harvesting index was found significant. The results showed that small seed size emerged earlier compared to medium and bold seeded variety. The reason may be due to small seed absorbs water quickly as compared to bold seeded and the breakdown of stored food material occurs resulting in plumule and radical growth quickly.[13] also reported that speed of germination is influenced by seed size in sorghum. The superiority of small seeds over large seeds may be attributed to its high speed of germination. Same results were reported in groundnut [14].

Foliar spray of nutrients and growth regulators also significantly influenced germination index of mung bean (Table 1). The highest germination index was recorded with the foliar treatment of 100 ppm indole butyric acid (18.3) and was significantly superior to rest of the treatments. Foliar treatments, 100 ppm salicylic acid, 2% NPK, 2% urea and 2% DAP recorded, 17.0, 16.3, 14.4, 17.0 and 13.1 germination index, respectively which differed significantly with each other while, the lowest germination index was recorded 12.3 seedlings/day without any foliar spray in control treatment.

The effect of seed size in relation to seed quality characters are warranted as amount of food reserve in seed is the basic requirement for its future expression in terms of emergence and final crop establishment. In the present investigation, bold seeded variety Pant Mung 5 showed significantly higher germination than other two varieties, medium seeded Pant Mung 4 and small seeded Pant Mung 2. The higher potential of bold seeded variety might be due to the initial capital and more amount of nutrients available for emergence as suggested by [15]. Gurbanov and Bertri [16] also stated that higher germination may be due to increased activity of redox enzyme in bold seeds which helps in quick breakdown of complex food into simple soluble sugars and also increase germination.

Among the foliar spray, the highest germination was recorded with the application of 100 ppm salicylic acid (82.8%), which was significantly superior to all the treatments except 2% urea (80.2%) spray. The lowest germination was recorded with control (74.4%) which was *at par* with 100 ppm indole butyric acid (75.3%), 2% NPK (76.8%) and 2% DAP (78.2%).

Maia *et al.* [17] reported that salicylic acid promoted germination percentage in soybean besides stimulating the length of the roots and increased green matter. Salicylic acid plays an important role to activate peroxidase enzyme which is required for biosyntheses of lignin and suberin that are involved in cell stress as these substances are of great importance for the protection of plants [18]. Kumar and Sarlach [19] also reported that there was considerable change in the seed germination with the foliar application of salicylic acid. It was reported that spray of salicylic acid control the transmission of fungal pathogen and help in improving quality seeds.

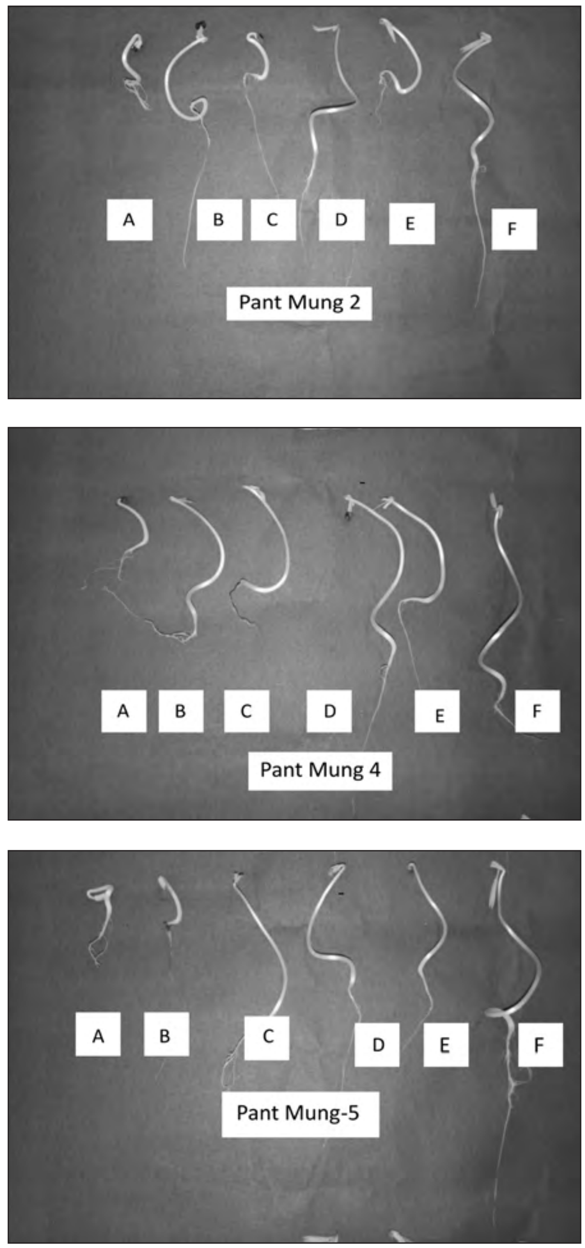

Plate 1. Effect of different varieties and foliar spray of nutrients and growth regulators (A) Control (B) Urea 2% (C) DAP 2% (D) IBA 100ppm (E) NPK 2% (F) Salicylic acid 100ppm on seedling vigour on mungbean seeds

Seedling length is important in terms of rapid germination and better establishment of seedling under field condition. Large seeds contain more nutrient reserve for the growth of resultant seedling. In the present study, bold seeded variety Pant Mung 5 produced longest seedling (14.0 cm/ seedling) as compared to small seeded variety Pant Mung 2 (10.6 cm/seedling) (Table 1 and Plate 1). Singh and Pai [20] reported that shoot and root length increased with increasing seed size in cowpea due to the reserve of high amount of carbohydrate in large seeds. Ali and Idris [21] also found that large seed size produced highest seedling length and seedling dry weight in fababean.

In foliar application of nutrient and growth regulators, 100 ppm salicylic acid produced longest seedling (16.4 cm/seedling) which was significantly superior to rest of the treatments. It was followed by foliar applied 100 ppm indole butyric acid (14.3 cm/seedling) and was statistically superior to the seedling length 12.7 cm/seedling and 12.6 cm/seedling recorded with foliar treatments 2% DAP and 2% urea, respectively which was *at par* with each other. The shortest seedlings were recorded with control (9.3 cm/seedling) and were *at par* with 2% NPK (10.0 cm/ seedling).

Seedling vigour index I (SVI-I) was computed by multiplying germination percentage and seedling length (cm/seedling). It was responded significantly by different varieties and foliar spray of nutrients and growth regulators. Data presented in table 1 indicated that the effect of varieties on seedling vigour index-I followed similar pattern to that described for germination percentage. The bold seeded variety Pant Mung 5 recorded highest 1113 value of seedling vigour index-I and was significantly superior to other two varieties, medium seeded variety Pant Mung 4 (986) and small seeded Pant Mung 2 (833). All the variety differed significantly with each other. Among the foliar spray, the maximum SVI-I was observed with foliar spray of growth regulator 100 ppm salicylic acid (1309) which was significantly superior to rest of the foliar treatments. Foliar treatments of 2% urea and 2% DAP were *at par* with each other with values attained 989 and 955 respectively. The lowest SVI-I was recorded with control treatment (679) and was *at par* with 2% NPK (782).

Seedling vigour index II (SVI-II) was computed by multiplying germination percentage and seedling dry weight (mg/seedling). It responded significantly to varieties and foliar application of nutrients and growth regulators. Among all the three varieties, the maximum SVI-II was obtained with bold seeded variety Pant Mung 5, (18.0) followed by medium seeded variety Pant Mung 4, (11.3). The lowest seedling vigour index-II was recorded with small seeded variety Pant Mung 2 (9.9). All the varieties differed significantly with each other. Vigour index is also used as a parameter of seed quality. Its potential can be determined in terms of seedling length, seedling dry matter and germination percentage. In the present study seedling vigour parameters namely shoot length, root length, seedling length, seedling dry weight, seed vigour I and II were significantly higher in bold seeded variety than medium and small seeded variety. The superiority of bold seeded over small seeded on vigour components may be attributed to its higher seed index, germination percentage, seedling length and seedling dry weight. The results obtained in the present study regarding seedling vigour parameters are in the agreement with the results reported in soybean [22].

The results obtained with different foliar spray of nutrients ranged from 10.5 to 15.9. In case of foliar application, 100 ppm salicylic acid recorded 15.9seedling vigour index-II which was *at par* with 100 ppm indole butyric acid (14.2) but differed significantly with rest of the treatments. The lowest SVI-II was recorded with control (10.52) which was *at par* with foliar spray of 2% NPK.Growth regulator showed significant effect on seed quality. Foliar spray of salicylic acid @ 100 ppm resulted in maximum seedling length, seedling dry weight and ultimately higher seed vigour index in bold seeded variety. It may be due application of salicylic acid which results in increased cell division within the apical meristem of seedling and enhanced root and shoot growth. These results are in line with [19] in cowpea**.**

Protein Content (%) and Enzyme Activity

The seeds of three different mung bean varieties produced with foliar spray of nutrient and growth regulators were also analyzed for protein and *á* enzyme and protease enzyme activity. In the present study it has been observed that bold seeded variety recorded significantly higher protein content than medium and small seeded variety. Supporting evidence was reported by [15]. They showed that protein content increases with increase in seed size.

In the present study, foliar application was done at flowering (40 days after flowering) and pod initiation stage

(55 days after flowering). The effect of foliar spray of nutrients and growth regulators with respect to protein content ranged from 19.7% to 22.1%. Protein content was significantly highest with foliar application of 2% urea (22.1%) because nitrogen is integral part of protein. It was followed by 100 ppm salicylic acid (21.6%) and 2% NPK (21.5%) which was *at par* with each other (Figure 1). The lowest protein per cent was observed in control (19.7%).Foliar spray of nitrogen in the form of urea increase nitrogen availability probably due to increase in nitrate reductase enzyme leading to synthesis of high amount of protein. Palta *et al.* [23] also reported that foliar spray of urea increases the protein content in seed due to increase in nitrogen availability for seed-filling in chickpea.

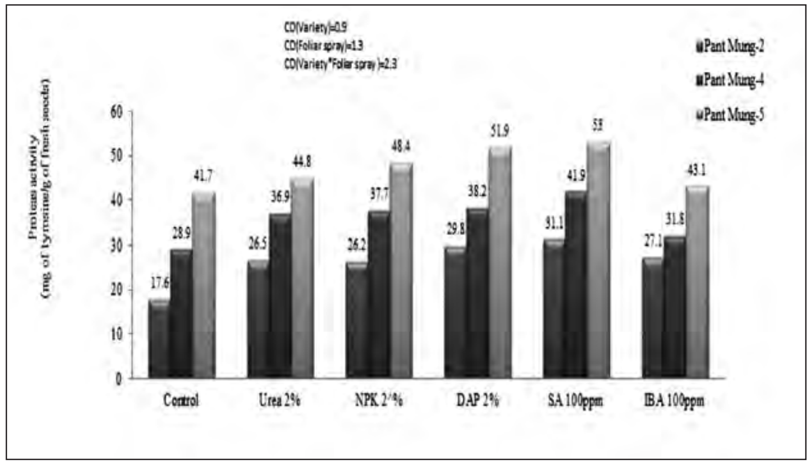

Figure 2. Protease activity of mung bean seed as influenced by varieties and foliar spray of nutrients and growth regulators

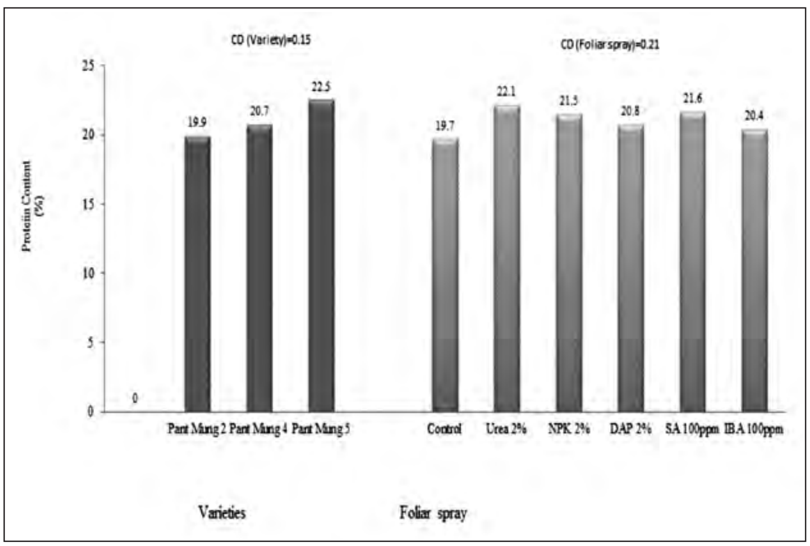

Figure 1. Protein content in seed in response to different varieties and foliar spray of nutrients and growth regulators

Amylases are also the key enzymes that play a vital role in hydrolyzing seed starch reserve thereby supplying sugar to the developing embryo Anuradha *at al*. [15] reported that large seeds had maximum α amylase activity due to higher metabolic activity for germination of seeds while due to unsuitability of small size, it was the lowest with small sized seed. The α amylase values recorded with foliar spray of nutrients and growth regulators ranged from 9.5 to 11.5 mg of starch hydrolyzed/g of seeds (Table 1). The highest activity was found with 2% NPK foliar application (11.5 mg of starch hydrolyzed/g of seeds) whereas the lowest activity was recorded with control treatment (9.56 mg of starch hydrolyzed/g of seeds). However, the differences were non-significant

The data in figure 2 showed significant effect of interaction of varieties and foliar spray of nutrients and growth regulator on protease activity (mg of tyrosine/g of seeds) in mung bean. Bold seeded variety Pant Mung 5 recorded maximum protease activity in comparison to small seeded variety Pant Mung 2 and medium seeded variety Pant Mung 4 in all the treatments. The interaction between bold seeded variety Pant Mung 5 and foliar application of 100 ppm salicylic acid (53.0 mg of tyrosine/g of fresh seeds) being *at par* with 2% DAP (51.9 mg of tyrosine /g of fresh seeds) registered significantly higher protease activity than remaining treatments.

Thus, it is concluded that, protein content in seeds increased significantly with 2% foliar spray of urea whereas, other quality parameters such as germination, percentage, seedling length, seedling dry weight, SVI-I, SVI-II, α amylase and protease enzyme activity were significantly highest in foliar spray of 100 ppm salicylic acid. Since these studies are based on one year experiments. Therefore, further study is required to reach a definite conclusion and recommendation.

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