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# Effect of Seed-Pretreatment with Some Plant Growth Regulators on Germination, Growth and Yield of Cowpea (*Vigna sinensis* Endl.)

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**Abstract** : Pretreatment of seeds of cowpea (*Vigna sinensis* Endl.) with indole-3-acetic acid and indole butyric acid had beneficial effects on their germination, dry matter production, flowering and yield. Treatment with Gibberellic acid resulted in increased rate of germination and plant height while the plants raised from seeds treated with distilled water were the same as the untreated plants (control) in terms of the measured parameters. Maleic hydrazide, on the other hand, had inhibitory effects on the rate of germination, dry matter production, flowering and yield. Neither indole-3-acetic acid nor gibberellic acid nullified the inhibitory effects of maleic hydrazide.
**Key words** : Flowering, Germination, Gibberellic acid, Indole butyric acid, Indole-3-acetic acid, Maleic hydrazide, *Vigna sinensis* Endl., Yield.

**植物生長調節物質による種子の処理がササゲ（*Vigna sinensis* Endl.）の発芽，生長，および収量に及ぼす影響**：J.C. オボンナ，P.G. アブラハム，（ジョス大学，植物学科）

**要　旨**：ササゲ（*Vigna sinensis* Endl.）の種子にインドール３酢酸またはインドール酪酸の前処理を施したところ，乾物生産性，種子収量，および発芽や花成の割合と時期などに対して有益な効果が見られた．ジベリン処理により発芽速度と主茎長が増加した．また，蒸留水を用いて同様に処理してもその効果は見られなかった．マレイン酸ヒドラジドは乾物生産性，収量，および発芽や花成の割合や時期などに対して阻害作用を示した．マレイン酸ヒドラジド処理と同時にインドール３酢酸やジベレリン処理を行っても，その阻害作用は消去されなかった．
**キーワード**：インドール３酢酸，インドール酪酸，花成，収量，ジベレリン，*Vigna sinensis* Endl.，マレイン酸ヒドラジド．

Cowpea is an important pulse crop in the tropics and subtropics particularly in Africa where it is grown for its long immature pods or dry seeds. Investigation on its yield improvement is very important because it has a short life span (3 to 4 months) and contains a high percentage protein (24%), which is deficient in most of the African staple foods.

Several reports on the regulatory effects of phytohormones on plant growth and development show that some of them can be used to enhance crop yield[3,4,27]. Although most of these experiments were based on the application of the hormones to the growing plants, many investigations showed that pre-sowing treatment of seeds with phytohormones could lead to increase in tissue hydration, redistribution of nutrient reserves, higher respiratory activities, and in consequence enhancement of seedling growth, dry matter production, early flowering and yield.[1,2,7,25].

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In this paper, the effects of pre-sowing treatment of cowpea seeds with indole-3-acetic acid (IAA), indole butyric acid (IBA), Gibberellic acid (GA), maleic hydrazide (MH), distilled water (DW) as well as the interaction between IAA and MH and between GA and MH on the germination, vegetative growth, flowering and yield are reported.

## Materials and Methods

### *1. Pretreatment media and procedure*

All the growth regulators were obtained from BDH Chemical Limited, Poore, England.

The media used for the treatment are IAA (10 ppm), IBA (10 ppm) GA (10 ppm), MH (20 ppm), IAA (10 ppm) + MH (20 ppm), GA (10 ppm) + MH (20 ppm), and distilled water. These concentrations were used because preliminary studies showed that high concentrations especially of IAA had inhibitory effects. Seeds without any treatment were used as the control.

One hundred grams of dry cowpea seeds were soaked in 40 m*l* of the treatment solution in a shake flask for 5 hours at room terperatur-

e. They were occasionally shaken for aeration and in order to keep them at a uniform moisture level. At the end of soaking, the excess solution was absorbed with a blotting paper. The seeds were air dried by spreading them over the blotting paper for 48 hours at room temperature (~28°C) so as to get the original dry weight of the seeds. The treatment was repeated two more times.

*2. Greenhouse and field experiments*

The effects of seed hormonization on the seedling growth was studied in a greenhouse. Ten randomly selected seeds from each treatment were germinated on a filter paper (whatman No.1) in glass petridishes containing 10 m*l* of distilled water. The filter papers were carefully changed every day and 5 m*l* of distilled water was added to avoid fungal infection. Dry weights of the seedilings were taken at intervals after drying at 60°C for 48 hours. These experiments were done for 5 days in three replicates.

A more detailed study on the growth, development and yield of the plants was conducted in the Botanical Garden of University of Jos, Nigeria. One hundred and sixty pots of equal size (i. d. = 50 cm) with a small hole at the bottom were arranged in sixteen lines of 10 pots each. The pots were filled with a mixture of soil and farmyard manure (3 : 1, v/v). Ten seeds from each set of pre-treated seeds and untreated control were sown in two lines of the pots. They were uniformly watered twice every day because the experiment was done during the dry season. After two weeks, the plants in each pot were thinned to six plants per pot.

*3. Measurements and statistical analysis*

The length of the main stem and number of primary branches were measured on ten randomly selected plants from each treatment every week. For dry weight measurement, 3 randomly selected plants from each treatment were carefully uprooted to minimize damage of the roots and wrapped in wet clothes before taking to the laboratory. The plants were thoroughly washed with tap water and then gently pressed between blotting sheets to remove moisture from the surface. Plant parts (roots, stems and leaves) were separated and the fresh weights were taken. They were oven dried at 60°C for one week and then weighed. Sampling was made every week.

ANOVA followed by the L.S. D test was used to find out the relative effects of each source of variance.

## Results

*1. Length of main stem*

There was a continuous increase in the length of main stem of the plants up to the 8 th week of growth after which it remained constant. Throughout the growth period, plants raised from the seeds treated with GA were taller than the rest of the plants. The main stems of the plants raised from the seeds pretreated with MH were shorter than those of the control plants (Fig.1). The effectiveness of each treatment is in the following order; [GA] > [IAA, IBA] > [DW, C] > [MH, IAA/MH, GA/MH] .

*2. Number of primary branches per plant*

From the 4 th week up to the 6 th week, there was no significant difference in the number of primary branches per plant raised from seeds treated with IAA, IBA and DW. However, from the 6 th week, the IAA- and IBA-treated plants showed the highest num-

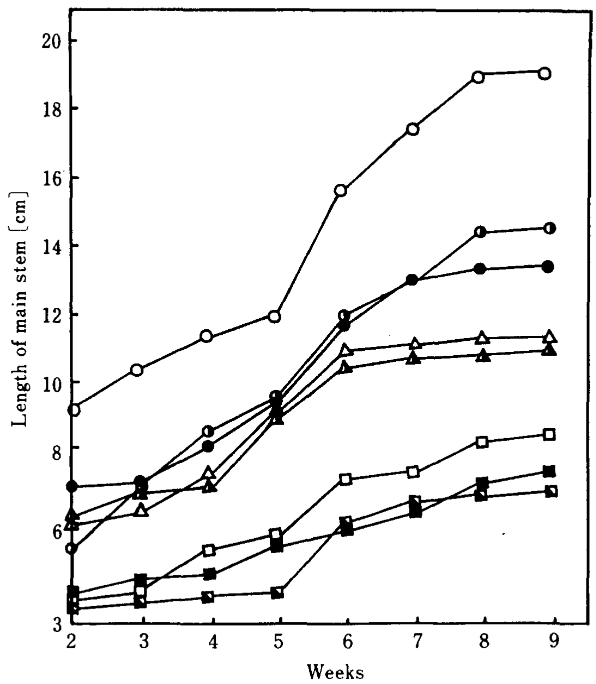



Fig. 1. Effect of pre-sowing treatment of seeds with indole-3-acetic acid (IAA, ◑), indole butyric acid (IBA, ●) gibberellic acid (GA, ○), maleic hydrazide (MH, □), IAA/MH (◪), GA/MH (■), distilled water (DW, △) and control (▲) on the elongation of the main stem.

ber of primary branches. GA and MH treatments resulted in a reduced number of primary branches when compared with the control. The least number of primary branches were found in the plants treated with IAA/MH and GA/MH (Fig.2). Statistically, this order is expressed as [IAA, IBA] > [DW, C] > [GA, MH, IAA/MH, GA/MH] .

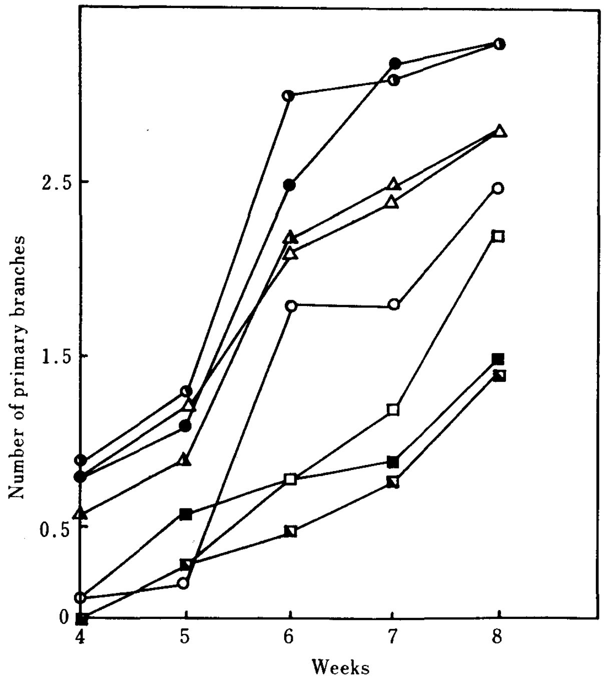



Fig. 2. Primary branch production as affected by presowing treatment of seeds with the plant growth regulators. Symbols are the same as in Fig. 1.

*3. Dry matter production*

The greenhouse experiments showed that the seeds treated with IAA, IBA and GA had better germination and seedling growth in terms of dry matter production than the control (Table 1). There was no difference in the rate of seedling growth between treatment with distilled water and the control. On the other hand, MH showed inhibitory effect. It is interesting to note that neither IAA nor GA could nullify this inhibitory effect. The results of the statistical analysis revealed that the order of effectiveness of each of the treatments is [IAA, IBA, GA] > [DW, C] > [MH, IAA/MH, GA/MH] .

The percentage germination of the seeds treated with MH, IAA/MH and GA/MH were so poor that only very few seeds germinated in the pots during the field experiments. As a result of this, measurements of dry weight in these treatments were not made. The few germinated seeds were left for yield analysis.

As shown in Figs. 3 A, B and C, pre-sowing treatment of seeds with IAA and IBA proved beneficial in accumulation of dry weight in roots, stems and leaves. There was no significant effect of pretreatment with distilled water while GA treatment resulted in slightly lower dry root and leaf weights.

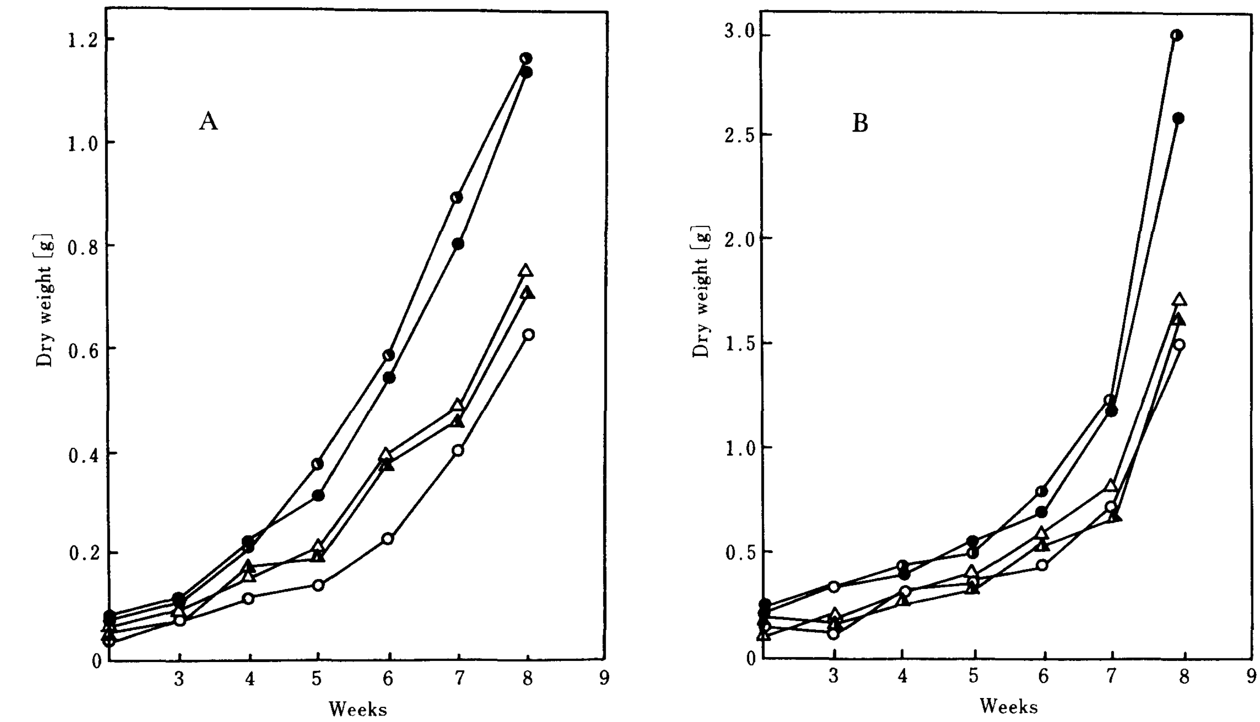
*4. Flowering*

As shown in Fig. 4, flowering was considerably made earlier in plants raised from the seeds treated with IAA and IBA. Consequent-

Table 1. Dry weight(g) of cowpea seedlings at various ages.

| | Time (days) | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 1 | 2 | 3 | 4 | 5 | Mean |
| IAA | 0.790 | 1.072 | 0.284 | 0.693 | 1.773 | 0.322 |
| IBA | 0.790 | 0.966 | 0.351 | 0.517 | 1.602 | 1.245 |
| GA | 0.917 | 1.128 | 1.231 | 1.489 | 1.611 | 1.275 |
| MH | 0.598 | 0.769 | 0.857 | 0.968 | 1.099 | 0.858 |
| IAA/MH | 0.576 | 0.657 | 0.770 | 0.865 | 0.963 | 0.766 |
| GA/MH | 0.512 | 0.731 | 0.775 | 0.864 | 0.963 | 0.769 |
| DW | 0.576 | 0.791 | 0.972 | 1.173 | 1.198 | 0.942 |
| Control | 0.554 | 0.787 | 0.995 | 1.238 | 1.246 | 0.964 |
| Mean | 0.664 | 0.863 | 1.025 | 1.226 | 1.264 | |

This experiment was done in a greenhouse.
The L.S.D. (0.05) for treatments, ages and interaction are 0.084, 0.073 and 0.206, respectively.
IAA : indole-3-acetic acid, IBA : indole butyric acid, GA : gibberellic acid, MH : maleic hydrazide, DW : distilled water.



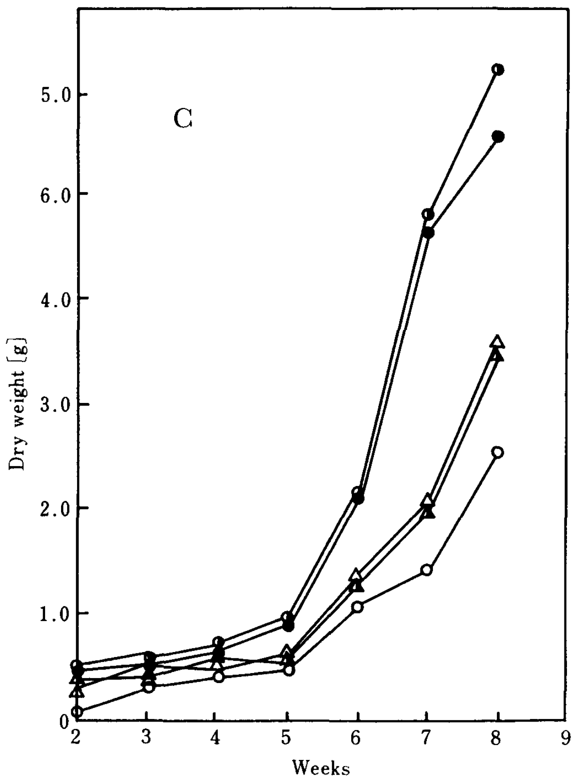




Fig. 3. Dry matter accumulation in plants raised from seeds pre-treated with different plant growth regulators. Symbols are the same as in Fig. 1. A : roots, B : stems and C : leaves.

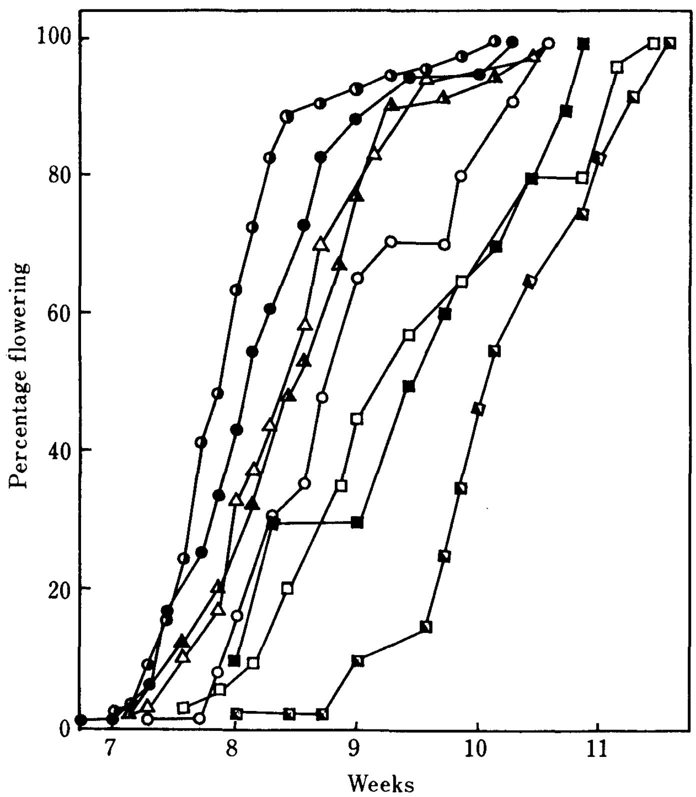



Fig. 4. Effect of pre-sowing treatment of seeds with plant growth regulators on flowering of the plants. Symbols are the same as in Fig. 1.

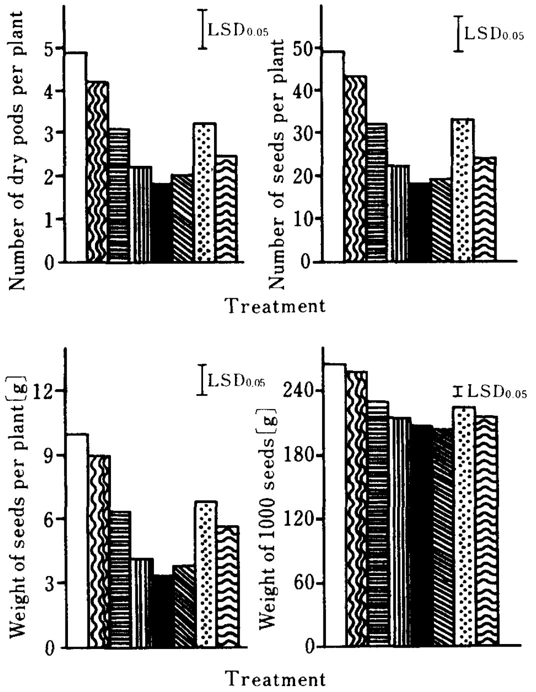



Fig 5. Effect of pre-sowing treatment of seeds with indole-3-acetic acid (IAA, □), indole butyric acid (IBA, ▧) gibberellic acid (GA, ▤), maleic hydrazide (MH, ▥), IAA/MH (■), GA/MH (▨), distilled water (DW, ▦) and control (▧) on the yield of the plants.

ly, 100% flowering was attained in these treatments within 10 weeks after planting. There was no significant effect of GA and DW treatments while treatment with MH, IAA/MH and GA/MH delayed the onset of flowering by more than 10 days. Consequently, some of the plants treated with MH, IAA/MH and GA/MH were still flowering even $11^{1/2}$ weeks after planting.

*5. Yield*

The effect of auxins on the number of pods per plant was highly significant. Plants raised from seeds treated with IAA and IBA produced the highest number of pods per plant. The effects of DW and GA treatments were not significant while MH, IAA/MH and GA/MH treatments resulted in the least number of pods per plant (Fig. 5.). L.S.D. analysis ranked the effects of the treatments in the following order; [IAA, IBA] > [DW, C, GA] > [MH, IAA/MH, GA/MH] . Similar trend was observed on the number of seeds per plant, weight of seeds per plant as well as weight of 1000 seeds from each treatment.

## Discussion

The results indicated that presowing treatment of cowpea seeds with IAA and IBA had remarkable beneficial effects on germination, seedling growth (Table 1, Figs. 1-3), flowering (Fig. 4) and yield (Fig. 5). Their stimulatory effects on plants have also been reported by many workers[5,20,22,25,29]. It is important to note that stimulatory effect of IAA is concentration dependent and most of the reported promotive effects of this phytohormone were obtained with low concentrations[21,22,29]. In preliminary experiments, we observed inhibitory effect on germination with concentrations of IAA higher than 50 ppm.

The exact mechanism of IAA action is still debated. According to the currently popular acid secretion hypothesis of auxin action[10], auxin stimulates growth by causing acidification of cell walls, which at low pH undergo an increase in extensibility that leads to rapid cell enlargement. This view is supported by many workers[14,30]. Morris and Arthur[21] reported that IAA stimulated an increase in the specific activity of acid invertase and concluded that the optimum concentration of IAA for both elongation and acid invertase activity was 10 uM. They further noted that IAA-induced promotion of acid invertase activity was not simply an indirect consequence of removal of end product (hexose) during IAA-induced cell growth and that a more direct action of IAA on enzyme turnover was involved. IAA could also act by increasing the uptake of water and solutes. Wheat seed pre-treatment with IAA and IBA increased the uptake of N, P,K and Ca by the seedling[7]. Osman et al[22] also reported increased uptake of P, N and Fe following treatment with IAA and GA. Apart from increasing the uptake of these minerals, their translocation could be increased by IAA treatment. Treatment with IAA resulted in increased sucrose uptake[19] as well as translocation[21].

From the above results, it can be concluded that IAA, at low concentrations, have stimulatory effects on crops. All of the above possible mechanisms can be acting jointly and any one of them may predominate depending on the environmental and other growth factors.

Although IBA had beneficial effects, the effect of IAA was always more pronounced. It has been shown that auxin having one bridging carbon atom between the nucleus and the carboxyl group as in IAA show the highest activity[26]. When the number of the bridging carbon atoms increases, there is generally a fall in the activity of the auxin. It is therefore, not surprising that IBA which has three bridging carbon atoms showed less activity. The difference in the structure could affect its availability at the site of action, rate of diffusion into the cells and tissues, rate of transport from the point of aplication as well as the rate of inactivation.

Pretreatment of seeds with GA had no significant effect on the yield (Fig.5). This conflicts with the results of Levy et al[18] who reported that foliar application of GA (280 mg/l) enhanced flowering, number and weight of capsules and the thebaine yield per plant of *Papaver*. GA was, however stimulating in both germination (Table 1) and the length of main stem of the plants (Fig.1). These results are in agreement with those of Pereira and Maeda[23] who reported beneficial effects of $GA_3$ on germination of *Vitis vinifera*. Fahmy et al[11] reported that while GA treatment increased germination in kenaf, it decreased it in roselle. This implies that crops could vary greatly in their response to the treatment with growth regulators. The exact mechanism of action of GA is not well known. A possible indirect action by stimulating the biosynthesis of other hormones has been reported[16]. This view is supported by the reports that treatment with GA resulted in an increase in the IAA level which was attributed to decreased conjugation of IAA with aspartic acid[12] or to lowered IAA-oxidase activity[28].

Although in the present investigation, pretreatment with distilled water had no significant beneficial effects, Kathiresen and Gnanarethinam[15] reported that sunflower seeds treated by 12 hour soaking in water and 12 hour drying produced vigorous seedlings under water stress conditions. Similar results were reported by Chhipa and Lal[7] as well as Chowdhury and Choudhuri[9]

Since presoaking treatments of seeds with water can have beneficial effects on the plants[7,9,15], the method of seed pretreatment may be more effective than direct application of the growth hormones to growing plants.

In all the parameters considered in this work, MH had inhibitory effect. This is consistent with the results of Helsel at al[13]. They reported that yield losses from application of MH to soybean occurred primarily as a result of decreased numbers of pods per plant.

Neither IAA nor GA could nullify the inhibitory effect of MH. However, Radi and Maeda[24] reported that GA application to the scutellum of rice reversed the inhibitory effects caused by application of brassinolide. The reason why neither IAA nor GA could reverse the inhibition caused by MH could be associated with the proposed mechanism of action of MH. The inhibitory action of MH could be through its action on the endogenous growth hormones. MH inhibited the action of endogenous IAA[17], and also inhibited the growth promoting action of GA in dwarf pea[6]. More investigations, using different ratios of concentrations of IAA to MH and GA to MH are needed before any conclusion can be made as to whether or not the inhibitory effect of MH can be overcome by the application of either IAA or GA.

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