HETEROSIS AND COMBINING ABILITY IN PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM* VIS L.)

BY

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A thesis submitted in partial fulfilment for the degree of

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other university.

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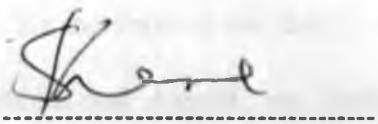

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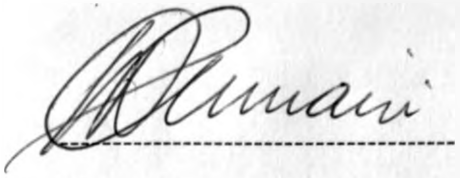

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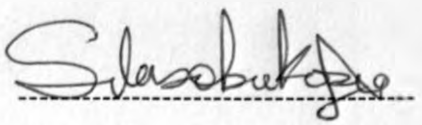

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DEDICATION

I dedicate my thesis first to the Almighty God, it was His will that I finish this work, second to the members of my family, my wife Pamellah, my children Ken and Lynn, my parents Andrea and Agness, my brothers Fred, Tofuko and Juma, my sisters Rosebellah and Scolasticka from whom I found love, moral, financial and physical support that enabled me go through my study.

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ABSTRACT

Development of F_1 hybrids in pyrethrum varieties offer a new opportunity to raise the declining yields of commercially grown clones. Requisites for hybrid varieties include effective pollination control methods and adequate levels of heterosis. This study was conducted to determine the extent of self and cross compatibility and estimate combining ability in six pyrethrum clones and levels of heterosis in their F_1 hybrids.

Self- and cross-compatibility tests were carried at University of Nairobi Faculty of Agriculture farm, Kabete (1820m). Self-compatibility was tested by repeated self-pollination of covered flower heads while cross compatibility was tested by precise timing and manual pollination of ray florets only (female only). The six parents and their F_1 were evaluated at Kabete (1820m) and Molo (2450m) for combining ability and heterosis. A complete 6 x 6-diallel analysis using Griffing's method 1 model 1 was used to estimate combining ability. Heterosis was estimated as percentage of mean performance of the hybrids above the better parent.

All the six clones were cross compatible. They produced viable F_1 seeds with germination percent range of between 5.7% to 30.8% pure germinating seeds and a mean of 18.4%. The six clones were self-incompatible. No viable seeds were produced after repeated selfing of the six clones. Seeds from the selfs were poorly filled, wrinkled, and dark in colour.

General combining ability (GCA) mean squares were significant ($P \leq 0.05$) for plant height, flower yield, pyrethrins content and pyrethrins yield in the two locations. Clone Ks/74 122 showed the best general combining ability for all the traits except plant height. Specific

combining ability (SCA) mean squares were significant ($P \leq 0.05$) for bush diameter, flower yield, pyrethrins content and pyrethrins yield in the two locations. Hybrids Ks/74/122 x L/64/4331, Sb/66/107 x L/64/4331 and Sb/66/107 x Ks 71/6 showed the best specific combining ability for pyrethrins yield with significant SCA effects in at least one of the locations ($P \leq 0.05$). GCA: SCA ratios were greater than 0.05 for all the traits except pyrethrins yields at Kabete. Plant height had the highest ratio of 0.89 and 0.79 while pyrethrins yields had the lowest of 0.46 and 0.55 at Kabete and Molo respectively impling that predictability of progeny performance based on GCA mean squares would be more accurate for plant height than for pyrethrins yields. Half of the hybrids had over 50% heterosis when compared to the better parent for pyrethrins yield. Hybrid Sb/66/107 x L/64/4331 had the highest heterosis for flower yield (132.9%) and pyrethrins yield (179.3%) while hybrid P4 x Ks/74/122 had the highest percent heterosis for pyrethrins content (27.3%) at Kabete.

It can be concluded from these results that self-incompatibility system strongly operates in the six-pyrethrum clones used in this study. The six-pyrethrum clones studied were cross compatible and produced hybrids that expressed heterosis in different agronomic characters of varied magnitude. For example, the observed significant heterosis for flower yield and pyrethrins yield appear to be interesting and can be exploited through hybridization. Flower yield, pyrethrins content and pyrethrins yields are under the influence of both additive and non-additive gene effects. They can therefore be improved through hybridization of superior plants in these characteristics followed by single plant selection.

CHAPTER ONE

INTRODUCTION

Pyrethrum (*Chrysathemum cinerariaefolium* Vis) is one of the Kenya's most important export crops. In 1991, Kenya exported 12,000 metric tonnes of pyrethrum that represented 80% of the world's output and earned US $ 23 million as foreign exchange (Pyrethrum Board of Kenya Annual Report, 1993). Pyrethrum is grown for extraction of insecticidal pyrethrins located in all plant parts but the ovaries of the flowers contains by far the highest concentrations and largest amount (Head, 1966; Brewer, 1973).

Pyrethrins have unique properties compared to synthetic insecticides. They have a high degree of suitability for combination with synergist (Chadwick, 1963), rapid knockdown, repellent and toxic effects for a great variety of insects (Van Rijn, 1974). They are practically non-poisonous to mammals (Griffin, 1973), have a rapid breakdown, therefore no persistence of residues and hardly any build-up of resistance in insect populations (Busvine, 1960; Fine, 1963). This allows pyrethrins to be used against insect pests in the house, treatments required just prior to harvest or stored food and livestock. Synthetic compounds resembling natural pyrethrins have been manufactured. Although these synthetics (known as pyrethroids) are more toxic to insects, their use is limited since they are environmentally unfriendly, toxic to mammals, have persistent residues, and insects develop resistance against them (Busvine, 1960; Fine, 1963). Although pyrethrum is essentially grown for pyrethrins extraction, it is also used as a cattle feed known as 'pyrethrum marc' (Griffin, 1974). Due to increased consciousness on the need to conserve the environment, the use of synthetic insecticides is reducing while the demand for natural pyrethrins based insecticides is growing. Pyrethrum production in Kenya has been declining all along from early 1990s (Figure1) despite the increase in demand. For

example in 1998/99. Kenya's quota for the world market was 10,000 tones but produced 3995 tones only (Pyrethrum Board of Kenya Annual Report, 1999). Pyrethrum production in Kenya is constrained by its agro-ecological requirements, low pyrethrins yield in the commercial clones used, land fragmentation in pyrethrum production areas due to population increase, high labour requirement, diseases and pests and stiff competition from other horticultural crops (Kroll, 1962; Glover, 1955; MacDonald, 1995).

Pyrethrum grows well in deep well-drained soils, preferably of volcanic origin. It requires some degree of chilling temperatures for flower bud initiation (Kroll, 1962; Glover, 1955). In Kenya, these environmental conditions are met in the highlands over 1800m above sea level, where pyrethrum production is confined. In these highlands, there is land fragmentation due to increase in population. This reduces the available land for pyrethrum production. The commercial clones grown have low pyrethrins yield per unit area with averages of about 1.5% pyrethrins content compared to 2.02% in Australia (MacDonald, 1995). Due to reduced land size, farmers are going for intensive agriculture. Priority is then given to high yielding crops that give high income (profit) per unit land or input. This has lead to many farmers abandoning pyrethrum production and going for high yielding horticultural crops.

Pyrethrum production requires a lot of labour. Planting, weeding, harvesting (picking flowers) and drying operations are carried out manually. It therefore means that most of the returns are used on hired labour and farmers end up with very little or no profit.

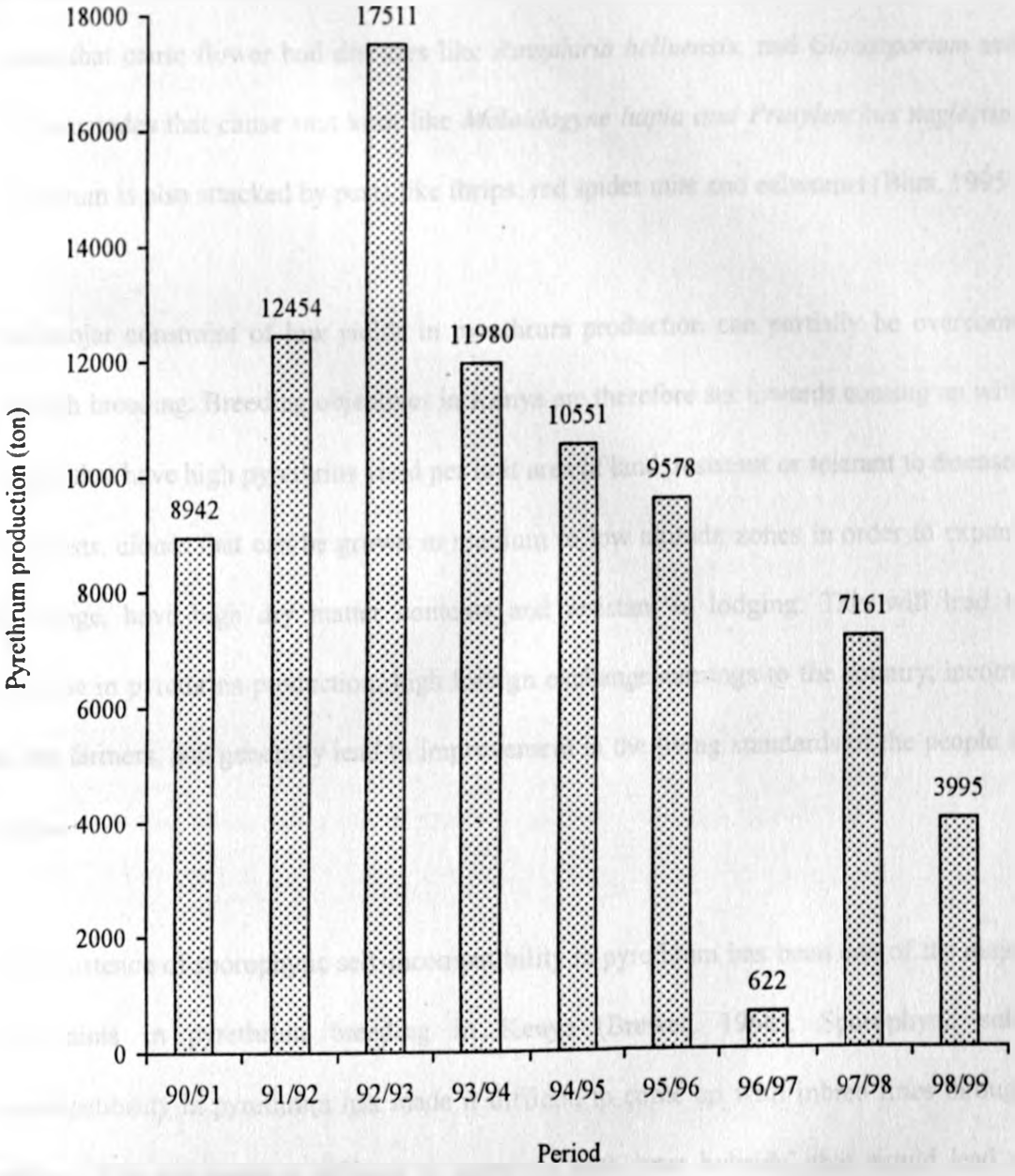


Figure 1. Pyrethrum production figures in Kenya from1990 to 1999
(Pyrethrum Board of Kenya annual report, 1999)

Pyrethrum production is greatly affected by diseases. The pathogens so far identified are the fungi that cause root rot like *Sclerotinia, Fusarium hytophthora, Pythium, Rhizoctonia, Thielaviopsis, Phoma, Stemphyllium, Colletotrichum,* and *Cylindrocarpun.* Those that cause flower bud diseases like *Ramularia belluensis,* and *Gloeosporium* and the nematodes that cause root knot like *Meloidogyne hapla and Pratylenchus neglectus.* Pyrethrum is also attacked by pests like thrips, red spider mite and eelworms (Bhat, 1995)

The mojar constraint of low yields in pyrethrum production can partially be overcome through breeding. Breeding objectives in Kenya are therefore set towards coming up with clones that have high pyrethrins yield per unit area of land, resistant or tolerant to diseases and pests, clones that can be grown in medium to low altitude zones in order to expand hectarage, have high dry matter contents and resistant to lodging. This will lead to increase in pyrethrins production, high foreign exchange earnings to the country, income to the farmers, and generally lead to improvement in the living standards of the people at large.

The existence of sporophytic self-incompatibility in pyrethrum has been one of the major constraints in pyrethrum breeding in Kenya (Brewer, 1968). Sporophytic self-incompatibility in pyrethrum has made it difficult to come up with inbred lines through selfing. This has made it difficult to come up with 'true hybrids' that would lead to maximum exploitation of hybrid vigor like it has been done in other crops like maize. Sporophytic self-incompatibility in pyrethrum has resulted in heterozygous and heterogeneous pyrethrum populations (Parlevliet and Contant, 1970). Selection and crossing for hybrid variety production from this population is hindered by the choice of

parents. The parents chosen should not be genetically related otherwise they cannot cross to produce viable seeds.

Some degree of self-compatibility has been reported in pyrethrum breeding program in Congo (Brewer, 1968). However, it is difficult to tell which clone is self-compatible. Before involving the selected parents in a hybridization program, there is need for prior testing for compatibility and combining ability.

It has been observed that in pyrethrum, there is inverse relationship between flower yield and pyrethrins contents (Parlevliet and Brewer, 1971). These two parameters are very important when considering improvement of pyrethrins yield per unit area. This make it hard to come up with clones that have both high flower yield and pyrethrins contents. Hybridization of different pyrethrum clones, has therefore potential for developing new clones with desirable traits. In order for hybridization program to succeed, there is need to determine whether it is possible to produce purelines that can then be used to produce "true hybrids". Purelines are produced mainly by repeated selfing of the selected parents to fix the desired traits. Since in pyrethrum contradictory reports exist in literature about self-compatibility, there is need to test for self-compatibility in order to determine the state of the parents to be used.

Due to the heterogeneous nature of pyrethrum population and the existence of sporophytic self-incompatibility, related clones couldn't be crossed to produce viable seeds. The breeding program should include crossing unrelated clones. The genetic potential of the parents to be included in the breeding program is of great importance. The parents should have high breeding value. It is necessary to obtain information on the genetic architecture

of the parents through combining ability tests. Parents that show high general combining abilities for the major agronomic traits should be included in the breeding program. Prior to development of pyrethrum hybrids there is need for information regarding the parental crosses that express high hybrid vigour for major traits.

The objectives of this research were: -

a) To determine the level of self and cross compatibility among the pyrethrum (*Chrysanthemum cinenariaefolium* Vis L) parental clone.

b) To determine level of heterosis in F_1 hybrids of pyrethrum (*Chrysanthemum cinenariaefolium* Vis L).

(c) To determine combining ability of the pyrethrum (*Chrysanthemum cinenariaefolium* Vis L) parental clones.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical aspects of pyrethrum

Pyrethrum (*Chrysanthemum cinerariaefolim* Vis L) belongs to the genus *Chrysanthemum* of the family Compositae. The genus is characterized by species that produce flowers with yellow ray florets. The genus contains more than 250 species of which three are of commercial value. Pyrethrum is a small, perennial, herbaceous flowering shrub reaching a height of approximately 80cm when mature. It has a fibrous root system that is concentrated in the top 30cm of the soil. The plant has rosette shaped deeply lobed leaves of variable shapes and length. The flower heads (capitula) are borne on branched leafy and slightly hairy stems whose base tends to become woody. It produces daisy-like flowers with bright yellow button center and narrow white petals (Parlevliet and Brewer, 1971).

The pyrethrum flower is an inflorescence consisting of an outer ring of white ray florets, which are female and the yellow disc florets which are bisexual [Figure 1(a)]. The ray florets, which are female, comprise of the ovary, style, stigma, calyx and white corolla [Figure 1(b)]. The disc florets possess a female pistil comprising of ovary, style and bilobed stigma and male filaments comprising of five filaments with anthers and stamen enclosed in a tube [Figure 1(c)]. The base of the corolla is tubular and engulfs a bilobed cylindrical style. The style is centrally placed in the floret on the superior ovary (Parlevliet and Brewer, 1971). On pollination pollen tubes grow through the conducting tissue in the center of the style. The cells of this tissue are somewhat elongated and terminated in the papillar of the receptive surface of the stigma.

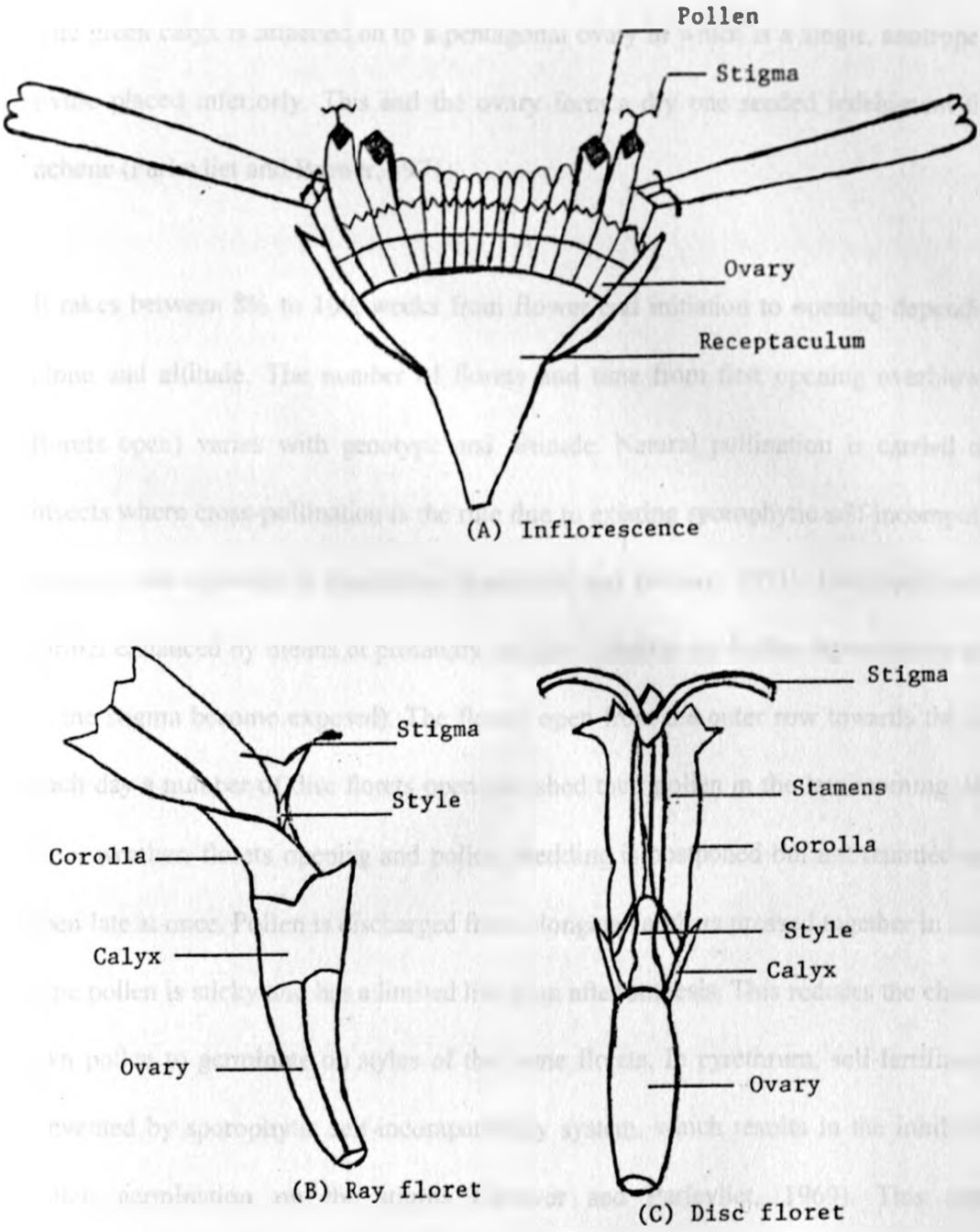


Figure 1: Structure of pyrethrum flower head, ray floret and disc floret.

The green calyx is attached on to a pentagonal ovary in which is a single, anotrope, erect ovule placed inferiorly. This and the ovary form a dry one seeded indehiscent fruit or achene (Parlevliet and Brewer, 1971).

It takes between 8½ to 10½ weeks from flower bud initiation to opening depending on clone and altitude. The number of florets and time from first opening overblown (all florets open) varies with genotype and altitude. Natural pollination is carried out by insects where cross-pollination is the rule due to existing sporophytic self-incompatibility systems that operates in pyrethrum (Parlevliet and Brewer, 1971). Cross-pollination is further enhanced by means of protandry (pollen is shed a day before the receptive surface of the stigma become exposed). The florets open from the outer row towards the center. Each day a number of disc florets open and shed their pollen in the late morning. During rainy weather, florets opening and pollen shedding is postponed but not retarded as they open late at once. Pollen is discharged from elongated anthers pressed together in clusters. Ripe pollen is sticky and has a limited life span after anthesis. This reduces the chance for own pollen to germinate on styles of the same florets. In pyrethrum, self-fertilization is prevented by sporophytic self-incompatibility system, which results in the inhibition of pollen germination on the stigma (Brewer and Parlevliet, 1969). This type of incompatibility has been observed to exist in other members of the same family Compositae such as *Pathenjum argentatum* (Garstel, 1950).

After successful pollination with pollen from a compatible clone, the styles dry up. It takes about 1½ months from pollination to seed maturity. Mature seeds are bright brown in color and easily fall off from the receptacle.

2.2 Pyrethrum production

Pyrethrum is cultivated almost entirely by small-scale farmers, currently numbering 50,000 and 60,000 with land sizes of between 0.25 to 1.0ha (Ikahu, 1987). They depend on the crop as their main source of cash income. The crop is also a major source of export revenue for the country, and this aspect provides gainful employment to over 3,500 additional workers. Pyrethrum is therefore of considerable economic and social benefit to Kenya.

The crop grows well at cool temperatures between 18^0C to 25^0C that occur in the higher altitude (1,800 to 2,900m above sea level) areas of Kenya. A minimum rainfall of about 1,000mm, evenly distributed throughout the year, is essential. Higher temperatures above 25^0C and dry weather have negative effects on flower yields and pyrethrins contents. Well-drained soils of moderate organic matter are ideal for pyrethrum cultivation (Parlevliet and Brewer, 1971).

New production fields in Kenya are established either from seedlings or from clonal propagates. A network of large-scale nurseries is maintained under the management of the Pyrethrum Board of Kenya to provide a continuous source of suitable planting materials. The nursery program is supplemented by propagation of superior clones through tissue culture. The commercial fields should be clean and a spacing of 45 x 45cm is used. During planting double super phosphate fertilizer is applied at a rate of 160 to 180kg/ha of P_2O_5 (Ikahu, 1987). The field is maintained clean by frequent weeding and earthing of the plants. First picking of mature flowers occurs within three to four months after transplanting, and thereafter at intervals of 10 to 14 days. Flower harvesting during the season is continued for up to 10 consecutive months, after which the stand is "cut-back"

to remove dead and unproductive plant material. The crop is then allowed to "rest", ready for renewed vegetative growth in the subsequent seasons. Flower harvesting is selectively done by hand, after which the flowers are suitably dried and delivered to the factory for processing (Bhat, 1995). Re-planting occurs during the fourth or fifth year. Pyrethrum is mainly cultivated for pyrethrins extractions that appear to be present in all plant parts. However only the flowers have content high enough for economical extraction.

2.3 Incompatibility system

Incompatibility is a form of infertility caused by the failure of plants with normal functional pollen and ovules to set seed upon pollination due to some physiological hindrance that prevent fertilization (Heslop-Harrison, 1971; Simmonds, 1979). This can be after crossing or selfing (Cross incompatibility and self-incompatibility respectively). Incompatibility can be categorized in to heteromorphic homomorphic. The later is associated with different flower forms on plants of the same species while the former is occurs in flowers with same flower structure of the same species (Watts, 1980). Homomorphic incompatibility can be either gametophytic or sporophytic in nature. Gametophytic and sporophytic homomorphic incompatibility are genetically controlled. Sporophytic homomorphic self- incompatibility is thought to be controlled by a multi-allelic locus (S-Locus). Some plants have a single S-locus while others more than one. In Brassica species that exhibit self-incompatibility, the S-locus has two alleles, SLG and SRK. The S locus is involved in the recognition reaction with self and non-self pollen. In cultivated radishes (*Raphanus sativus*), six alleles were identified at the S-locus tentatively named S201 to S206. (Hatakeyama *et al* 1998; Niikura and Matsuura, 1998; Watts, 1980)

In gametophytic homomorphic self-incompatibility operating in dicots, compatibility is determined by the 'S' alleles within the S-locus of the pollen. If the 'S' alleles in the pollen are identical with either of the alleles present in the stylar tissue, which is diploid, pollen tube growth in the style will be slowed and fertilization will not occur. However, if the 'S' alleles in the pollen grain are different from both the alleles in the stylar tissue, there is normal pollen tube growth and fertilization. For example, a plant with genotype S_1S_2 is pollinated with another plant with the same genotype the pollen nucleus will contain either an 'S_1' or 'S_2' alleles. Because these two alleles are present in the stylar tissue, fertilization will not occur. If the same plant receives pollen grains from another plant with genotype 'S_1S_3', the pollen grains will contain either 'S_1' or 'S_3' alleles; in this case pollen with 'S_3' allele only will develop pollen tubes and cause fertilization (Poehlman, 1987; Watts, 1980).

Sporophytic homomorphic self-incompatibility is determined by the alleles in the S- locus of the sporophyte part of the plant from which the pollen grains originated. In this case the alleles exhibit dominance. The plant producing pollen determines the dominance. If a plant has a genotype of 'S_1S_2' at the S-locus, and 'S_1' is dominant over 'S_2', then all the pollen from this plant will function as if it were 'S_1'. Pollen grains from this plant with either 'S_1' or 'S_2'allele at the S-locus will be incompatible with stylar tissues that bear an 'S_1' allele but will be compatible with styles with an 'S_2' allele at the S-locus. In sporophytic homomorphic incompatibility, hindrance to pollen germination or pollen tube is localized on surface of the stigma in contrast to gametophytic homomorphic incompatibility system in which hindrance to pollen tube growth is in the style (Poehlman, 1987; Heslop-Harrison, 1971; Simmonds, 1979; Watts, 1980).

In pyrethrum, sporophytic homomorphic self-incompatibility system strongly operates. Despite this system which prevent self-pollination, selfed seeds have been formed by forced-self-pollination (Brewer and Parlevliet, 1969). This has been done by covering the flower heads with cellophane bags thereby denying the plant any chance of open pollination. This type of selfing can be explained by the fact that older florets accept own pollen if foreign pollen is absent (Brewer, 1968). Due to the existence of sporophytic self-incompatibility in pyrethrum and the heterogeneity that occurs in pyrethrum populations, genetically similar clones cannot cross-pollinate to produce viable seeds (Brewer and Parlevliet, 1969).

After pollination, there is an exchange of exudates between the pollen grains and the stigma (Heslop-Harrison, 1971). Only pollen from compatible male parent remains stuck on the stigma. Due to the mismatch between the exchanged exudates, pollen from incompatible parent fall-off. The radishes (*Raphanus sativus*), the S-locus has been shown to carry a glycoprotein gene (SLG) which participates in the pollen-stigma interaction of self-incompatibility (Sakamoto *et al*, 1998). In cabbage (*Brassica oleracea*) Polyacrylamide gel isoelectric focusing was used to study the proteins in stigmas, a specific band was observed in the PI 8.3-8.5 region in self- incompatible lines. According to the characteristics of this band, it was concluded to be the S-locus specific glycoprotein band (Song-Ming; *et al* 1998). Heslop-Harrison (1971) working on Lily observed that, the exudates from the stigma promote pollen germination and growth through the papillae down the style. It has been determined that the amount of nutrients in the pollen tubes alone is not enough to provide for its nourishment up to the time it reaches the ovary. The main source of nourishment for pollen tube growth is therefore from the style wall. Pollen tubes absorb nutrients from the cells lining the style wall as they grow down. Secretions

from the cells lining the pistil canal help in pollen tube growth. The secretions have chemotropic effect by directing pollen tube growth downwards. They also have growth regulation and nutritional functions (Heslop-Harrison, 1971). Mismatches or differences in constitution and or concentrations of these exudates between two parents are the primary cause of incompatibility.

2.4 Pyrethrum breeding in Kenya

Pyrethrum is a diploid plant with 18 chromosomes (2n = 2x = 18). Due to the presence of self-incompatibility systems in pyrethrum, pyrethrum populations are highly heterogeneous and heterozygous. Breeding for pyrethrins yield is the major objective. However, pyrethrins yield per unit area of land is a function of many yield components that include number of flowers, flower size, and pyrethrin content of the dried flowers. These yield components are influenced by environment qualitatively and quantitatively (Tuikong, 1984).

Pyrethrum breeding started in Kenya in 1936 when the senior plant breeder at Njoro made selections from a commercial field and obtained high toxic clones. The selections were based on individual plants showing desirable features such as upright growth (resistance to lodging), vigor of growth and prolific flowering (Parlevliet and Brewer, (1971). The selected plants were reproduced vegetatively by repeated splitting. The clones produced were tested for pyrethrins content. The best selected clones were multiplied and planted in alternating rows on isolated field to produce 'hybrid seed' as selfing was practically excluded (Brewer and Parlevliet, 1969; Brewer, 1968; Kroll, 1962). From 1940 to 1965 pyrethrum-breeding program in Kenya was principally oriented towards the production of hybrids and synthetics. In 1942 the first hybrid variety called the 'high toxic' was released.

The selected parental clones were phenotypically dissimilar so the resulting seedling populations were highly heterozygous and heterogeneous. Selection for superior clones was however, further complicated by observed negative correlation between flower yield and pyrethrins content. As a result of this, individual plants with high flower yield and a high pyrethrins content were very rare. Selection of high flower yielding individual plants as parents of hybrid seed was ineffective, as this did not necessarily give superior progenies. It was not until in the late 1950s when it was realized that some clones were good enough to be grown commercially. Asexual multiplication of superior clones was considered a better method of improvement than the production of hybrids (Brown, 1965; Glynne, 1968; Drain and Shuey, 1934; Parlevliet and Contant, 1970).

Breeding, selection and commercial vegetative propagation of outstanding clones was started at National Pyrethrum and Horticultural Research Center at Molo in 1962 [Contant, 1963(a)]. This scheme was simply mass selection where phenotypically outstanding plants were selected, split and planted in single lines for a year. The promising clones were then evaluated in replicated yield trials, for 2 to 3 years. Contant [1963(b)] however realised that desired genetic characteristics in a population could be improved in a fairly shorter time if promising clones were tested for their breeding values (BV). He therefore introduced the polycross test. The use of the polycross test for assessing the BV of individual plants, clones or inbred lines is based on the assumption that with adequate randomization, all plants in the polycross plot receive approximate the same compound pollen mixture. The differences among their progenies apart from environmental effects are therefore indicative of the BV of the seed-producing parent. Genetic characteristics of selected plants could then be preserved by vegetative propagation (Parlevliet and Contant, 1970). Clones with high BV were crossed in pairs to

produce hybrids that were tested as described by Kroll (1962). The polycross method was used to select clones that produce superior hybrids when crossed with other clones that have good general combining ability (GCA) (Parlevliet and Contant, 1970). A hybrid variety P4, which was developed by mixing three clones with high GCA for yield of pyrethrins per hectare, was released in 1970.

Two selection schemes are currently used at National Pyrethrum and Horticultural Research Center in Molo for population improvement. These are clonal breeding (selection) and variety breeding (generative breeding). The two programs are interrelated. The main features of each program are discussed below.

A) Clonal breeding:

It is the selection of promising single plants from a genetically variable population of pyrethrum seedlings followed by splitting and subsequent agronomic and yield potential assessment over several years and different location. It has five steps:

Step 1: Single plant selection: Seedlings of a good variety are planted at six selected locations. These seedlings form a genetically variable population from which individual plants are selected. At the end of the year, out of about 15,000 seedling planted, 500 upright single plants which are healthy, vigorous and prolific in flowering are selected at each site to enter the next step.

Step 2: Single line observation trial: Each selected plant is divided into 12 splits to form single line (clones). A commercial clone is used as a control. The control clone is planted after every 40 lines. The poor lines are discarded and the well-established ones are

sampled twice for pyrethrins content. At the end of the final selection, healthy, vigorous, abundantly flowering lines with a pyrethrins content of at least 1.8% are selected to enter the next step.

Step 3: Screening trial: Each selected line is further split to give a single plot of 60 plants. Normally around 60 lines out of 500 are selected at each site and these are inter-planted with plots of the control after every sixth plot. All clones are measured for pyrethrins content and the flower yield recorded at every picking. At the end of the year and on the basis of continuous phenotypic assessment, superior clones are selected to enter the next step.

Step 4:Replicated yield trial: The selected clones from the screening trial are split to give three plots of 60 plants each. About 11 clones from screening trials are selected at each site. The flower yields are taken at the end of two years.

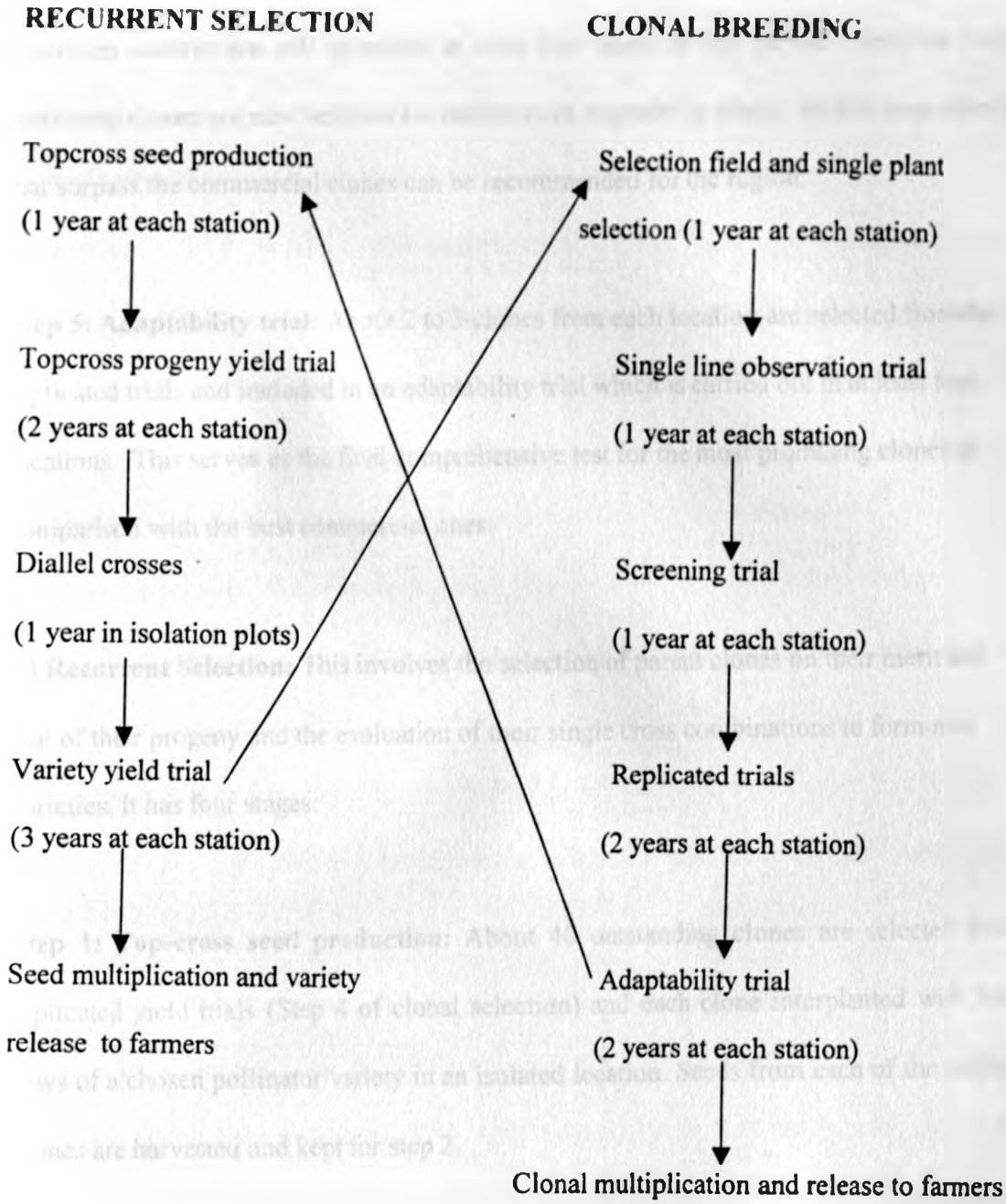


Figure 3: Pyrethrum breeding cycle currently practiced at National Pyrethrum and Horticultural Research Center, Molo (Pyrethrum Board Annual Report, 1995).

Pyrethrins content are still measured at least four times in this period. Only the very promising clones are now selected for inclusion in adaptability trials. At this stage clones that surpass the commercial clones can be recommended for the region.

Step 5: Adaptability trial: About 2 to 3 clones from each location are selected from the replicated trials and included in an adaptability trial which is carried out in at least four locations. This serves as the final comprehensive test for the most promising clones in comparison with the best commercial ones.

B) Recurrent Selection: This involves the selection of parent clones on their merit and that of their progeny and the evaluation of their single cross combinations to form new varieties. It has four stages:

Step 1: Top-cross seed production: About 40 outstanding clones are selected from replicated yield trials (Step 4 of clonal selection) and each clone interplanted with four rows of a chosen pollinator variety in an isolated location. Seeds from each of the mother clones are harvested and kept for step 2.

Step 2: Top-cross progeny trial: The top-cross progeny seedlings raised in step 1 are planted out in replicated yield trials at various locations and on their performance, the clones with good general combining ability are chosen for a diallel cross

Step 3: Diallel cross: Up to eight mother clones are chosen and a diallel cross made between them. Usually reciprocal crosses are excluded to limit the number of isolation plots.

Step 4: Variety trials: The progenies of the diallel cross are evaluated for their yield of pyrethrins per hectare in replicated trials at various locations. Usually the control used is the highest yielding variety so that the hybrids could be compared.

Ndambuki (1979) reported that diallel cross method was the most efficient in determining combining ability in pyrethrum. This method however is not being used in parental screening because it is laborious. At the National Pyrethrum Research Centre, Molo, polycross method is used for parental screening and diallel cross used only in clonal selection. Employment of diallel cross method using precise manual crossing in screening of parental clones has not been reported. In all crossing activity undertaken in pyrethrum breeding in Kenya, pairwise planting of pyrethrum parents in isolated plots is used. This method assumes that there is full self-incompatibility and cross-compatibility among the pyrethrum parental clones. Production of pyrethrum varieties (hybrids) has for a very long time depended on a few chosen pyrethrum mother clones (Ikahu and Ngungi, 1988).

It is therefore necessary to develop a reliable screening method that can be used in screening many more materials. Those clones found to be compatible and have good combining ability are then used as mother clones. This leads to enhancement of genetic diversity. Pyrethrum breeding and production is also being carried out in other countries around the world. Some of these works had very encouraging results from which Kenya can emulate to improve its pyrethrum production.

2.5 Pyrethrum production and breeding in other countries

The pattern of world pyrethrum production has been greatly influenced by the effect of World wars. Dalmatia was the main source of pyrethrum up to World War I, after which

Japan took over. Following the outbreak of World war II. Japan ceased to be a significant producer and Kenya overtook it. There had been various attempts to introduce or expand the growing of pyrethrum in other parts of the world. Many of these attempts did not prove economical and were discontinued (Wainaina. 1995).

Over the past decades, major efforts in development of commercial pyrethrum clones have been made. However, significant improvement has been achieved only in East Africa (Kenya and Tanzania) and in Australia (Gullickson. 1995). Pyrethrum production in Australia dates back to 1890. In 1931, the Plant Industry Division introduced four pyrethrum strains from Dalmatia. Yields from the four strains were between 1200 to 1400 kg/ha of dry flowers and pyrethrins content of between 0.8 to 1.0%. Seeds from these strains were harvested and given to farmers. However, the crop developed from these seeds did not perform well. The flower yield was very low with pyrethrins contents of about 0.22% and was severely affected by Fusarium root rot. These constraints led to the abandonment of the crop. Another attempt was made in 1944 during World War II. Minor improvement was made but it was abandoned due to introduction of DDT and increased supply of pyrethrum from Kenya (Gullickson, 1995).

Meaningful and serious commercial production of pyrethrum in Australia started in 1979. Seeds were brought in from Kashmir, India. Initially these seeds were grown in greenhouses where selection was carried out (Gullickson. 1995). Superior clones and seeds in multi-location yield trials were released in 1981. The seeds had mean pyrethrins content of 1.92% while the best clone had 2.16% against 1.33% in the unselected base population. Flower yields were also high in improved clones. Commercial production of the crop from seeds was not favorable due to the cross-pollinating nature of the crop that

The clones released for cultivation were selected for high flower yield, pyrethrins contents, lodging resistance, uniform plant canopy characteristics, and synchronous flowering. This led to the production of "Hypy" pyrethrum cultivar which had pyrethrins content of 2.02%, dry flower yield of over 2400kg/ha and pyrethrins yield of 48.8kg/ha. This was the world's first patented cultivar of pyrethrum. Vegetative propagation techniques through tissue culture, mist propagation of stem cutting were developed and perfected at the University of Tasmania (Gullickson, 1995). These techniques were all employed together to increase the number of plants of any selected genotype in the shortest possible time (MacDonald, 1995; Gullickson, 1995).

In Tanzania, the Pyrethrum Board of Tanzania carries out pyrethrum improvement program. The program entails both varietal and clonal breeding similar to that carried out in Kenya described above (Wainaina, 1995). Pyrethrum production in United States of America was in two phases. The first phase started in 1877 by Milko using seeds from Dalmatia. The seeds were grown as commercial crop in Stockton (Gullickson, 1995). This expanded to Merced County where over 1400 acres were under pyrethrum. Pyrethrum was used to produce pyrethrum powder by the name "BUHACH". This phase collapsed in 1940s. The second phase started in 1987 when Dr. Bhat was contracted by John I. Haas Company to work on newer strains of tissue cultured clones. He developed high yielding clones. However the Haas Company kept the details of his program confidential (Gullickson, 1995). A combination of good selection and hybridization techniques, tissue culture and polyploidization has lead to great achievement in pyrethrum breeding and production in some countries.

2.6 Breeding through polyploidization

The haploid chromosome number in pyrethrum is nine. The cells in vegetative parts carry the diploid number of 18 chromosomes. Polyploids in pyrethrum occur spontaneously and can be induced by use of mutagens such as colchicine. Spontaneous occurrence of triploids has been known since 1950's. Contant first undertook artificial induction of polyploids in Molo in 1962. Some of the polyploids were analysed again by Brewer in 1968. Studies on polyploidy in pyrethrum have indicated that it may result in improvement of morphological characteristics of the plant. Ottaro (1977) observed that triploids in pyrethrum were taller with increased flower diameter and flower weight compared to diploids and tetraploids. Polyploidy has been used on limited scale in pyrethrum since it provides no significant advantage over other breeding methods (Bhat, 1995). For Kenya to realise good achievement in pyrethrum breeding, selection of parents that have high combining ability for hybridization still remains the main method for pyrethrum crop improvement.

2.7 Combining ability and diallel analysis

Spraque and Tatum first used the term combining ability in 1930s during the development of hybrid maize (Simmonds, 1979). Combining ability can be divided in to two, general combining ability (GCA) and specific combining ability (SCA). GCA is used to designate the average performance of a line in hybrid combinations while SCA designates those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the line involved (Spraque and Tatum 1943). GCA is therefore the main effect and SCA is an interaction. In plant breeding, combining abilities have found their main use in predicting performance of hybrid population of outbreeders although they are also applicable to inbreeders.

Combining ability (both GCA and SCA) in pyrethrum can be estimated using four different methods namely, diallel (single crosses), top cross with high yielding tester, top cross with low yielding tester and polycross methods (Ndambuki, 1979). Diallel cross was reported to be the best method of determining GCA and SCA in pyrethrum. A diallel cross is a set of all possible mating combinations between several genotypes which may be defined as individuals, clones or homozygous lines (Hayman, 1954). The information and interpretation of data from a diallel cross depends on the assumption made and whether or not parent, F_1, and reciprocals are included. Two models are usually used. Model I (fixed effects), the experimental material is regarded as population about which inferences are made. The objectives are to compare combining abilities of parents, when the parents themselves are used as testers and to identify the high yielding combinations. In model II (random effects), the assumption is that the study material constitutes random samples from some parent population and inferences are not to be made about the individual lines but about the parameter in the parent population. The particular interest in this case is the estimation of genetic and environmental components of complex population variance. It is assumed that the effects in the model (except the population mean) are normally and independently distributed, with means zero and variance δ^2_l where l denotes general or specific combining ability, block effect or reciprocal effects. The method is used to investigate general properties and evaluate the performance of parents and crosses in plant breeding programs. Diallel cross method can be used to investigate reciprocal differences, heterosis, genotype x environment interaction and mode of gene action (Jinks, 1954).

In a diallel analysis average performance of each progeny is broken into components relating to general combining ability (main effects) and into specific combining ability (interaction). If SCA mean square is not significant, then performance of a single cross progeny can be predicted on the basis of GCA. Crossing the two parents having the highest GCA may produce the best performing progeny. In cases where SCA mean square is significant, total genetic variance among single cross progeny performance is then determined by estimating the component of variance and expressing them in the ratio of two times the variance due to GCA over total variance (variance due to GCA plus SCA). The closer this ratio is to unity, the greater the predictability based on GCA alone (Baker, 1978).

That is;

$$\frac{2\delta^2_{gca}}{2\delta^2_{gca} + \delta^2_{sca}}$$

In a diallel crossing system, the additive and non-additive components of the parent genotypic variance are estimated as GCA and SCA effects and or variances respectively. Griffing (1956a) defined GCA and SCA effects and variances and their relationship to additive and non-additive genetic effects and variances as follows: -

$$\delta^2_G = \delta^2_A + \delta^2_D + \delta^2_I$$

However, $2\delta^2_{gca} = \delta^2_A + 1/4\delta^2_I$

And $\delta^2_{sca} = \delta^2_D + (\text{residual } \delta^2_I)$

Therefore, $\delta^2_G = 2\delta^2_{gca} + \delta^2_{sca}$

Where; - δ^2_G = Population genotypic variance

δ^2_A = Additive genetic variance

δ^2_D = Dominance variance

δ^2_I = Total epistatic variance

δ^2_{gca} = General combining ability variance

δ^2_{sca} = Specific combining ability variance

The dominance (δ^2_D) and epistatic (δ^2_I) variance comprise the non-additive (δ^2_{NA}) genetic variance. In diallel analysis, the models and theories given shows that the technique is an important tool in finding out the mechanisms of gene action in inheritance of certain traits. The analysis is mainly used in studying quantitative traits that exhibit complex modes of inheritance. Performance of crosses thus can be forecasted by employing combining abilities. Therefore the significance of combining abilities is that they provide an empirical summary of complex observations and a reasonable basis for forecasting the performance of untested crosses but still make no genetical assumptions (Simmonds, 1979).

Contant [1963(b)] introduced combining ability in pyrethrum after realizing that genetic improvement of a population could be improved in a fairly short time if the clones were first tested for combining ability. He introduced the polycross method. It was by this method of testing for combining ability that hybrid variety P4 was developed and released in 1970.

Parlevliet and Contant (1970) determined breeding value (GCA) in 22 clones and 33 single crosses of pyrethrum using polycross method. This was determined by heritability.

which was estimated from the regression of the polycross progeny data on those of the female parent clone. It was determined that for flower yields, pyrethrins contents, flower size and resistance to lodging, their actual values were highly correlated with the estimated values calculated from the breeding values of the parent clones. For dry matter contents, there was no correlation between the parents and progenies. It therefore shows that for flower yield, pyrethrins contents, flower size and resistance to lodging, performance of single crosses is caused predominantly by the general combining ability of the parent clones. Similar results were obtained by Ndambuki (1979) using different combining ability test methods. However, Bhat (1995) reported that a 5 x 5 diallel cross- carried out by Pandita (1983) had their SCA greater than GCA. Currently polycross method for testing for GCA is still being employed due to its simplicity (Ndambuki, 1979). The pyrethrum parental clones with high GCA should be selected and used in hybridization program. This would be of value only if the traits required to be improved on express hybrid vigour (heterosis).

2.8 Heterosis

Allard (1999) described heterosis as the manifestation of greater vigor in height, leaf area, growth, dry matter accumulation and higher yield in the F_1 hybrid in comparison with its inbred parents. It has been regarded as the converse of the deterioration that accompanies inbreeding. Since all the beneficial effects of crossing are manifested in F_1 hybrids, hybrid vigor has always been emphasized more than inbreeding depression (Allard, 1999). Many theories have been put forward to explain the cause of heterosis. These include the dominance hypothesis, overdominance, physiological stimulus, complementation at cellular and sub cellular level, balanced metabolism, hormonal and other factors (Allard, 1999; Suresh and Renu, 1975). Despite the many theories put forward to explain

heterosis, present concept of heterosis has no clear cut-qualifying hypothesis. This has led to the absence of a direct relationship between gene and complex phenotypic expressions always recognized in interpretation of quantitative characters (Allard, 1999; Suresh and Renu, 1975).

Heterosis (hybrid vigor) has been exploited very much in many crops. It finds it's advantage from the fact that various desirable genes in different parents (inbred lines) are brought together in the F_1 plants. Due to the combination of the desirable genes in the hybrid, it performs better than two parents. Like in other crops, Singh and Sharma (1989) determined that heterosis in pyrethrum was positively correlated to selection divergence. Heterosis was high when the two parents used were highly diverse in characters to be improved on. Development of genetically diverse inbred parents for hybrid production in pyrethrum is hindered by the existence of sporophytic self-incompatibility. Despite this difficulty, he determined that selecting diverse parents from pyrethrum population could achieve heterosis of about 80% for pyrethrins yields and 97% for number of flowers per plant. Heterosis has been exploited in pyrethrum along since the start of pyrethrum breeding in Kenya. This has been through selecting parents from the population followed by crossing them to produce hybrids. From the hybrid population plants with outstanding performance (those that perform better than the parents) are selected. It was through this method that hybrid variety P4 was released in 1970 (Ikahu and Ngugi, 1988).

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

Two experiments were undertaken during this study. The first experiment was designed to determine the levels of cross- and self-compatibility among and within six parents. This experiment was carried out at the University of Nairobi, Faculty of Agriculture farm, Kabete Campus Field Station under controlled pollination between January and December 1995.

The second experiment was carried out to determine heterosis and nature of gene action in crosses involvig six parental clones. This experiment was carried out at Kabete field station and at National Pyrethrum and Horticultural Research Center, Molo between January 1996 and June 1998.

3.2 Materials

Six pyrethrum clones (*Chrysanthemum cinerariaefolium* Vis) were selected on the basis of their proven performances in flower yields, pyrethrins content and vigour. A brief description of the parental material is given in Table 1.

3.3 Experimental sites

Kabete Field Station is in Nairobi district (Nairobi Province) in Kenya. It lies on latitude 1° 14' 20" S and longitude 36° 45' E. Kabete field station falls within the upper midland zone at an altitude of 1, 820m above sea level on the average (Jaetzold and Schmidt, 1983).

Table 1. Characteristics of six pyrethrum parental clones

Parental clone	Characteristics
1) L/64/4331	Released for commercial growing in areas with altitude over 1800m above sea level. It has a flower yield ranging from 1000 to 1200kg/ha/year and pyrethrins content of 1.6% on average.
2) Sb/66/107	Released in 1976 for commercial growing in areas with altitude over 1700m above sea level. It has a flower yield of between 900 to 1000kg/ha/year and pyrethrins content of 2% on average.
3) Ks/71/6	Released in 1978 for commercial growing in areas with altitude above 1700m above sea level. It has a flower yield of 900-1000kg/ha/year and pyrethrins content of 1.7% on average.
4) Ks/70/64	Released in 1979 for commercial growing in areas above 1700m above sea level. It has flower yield of 1000-1100kg/ha/year and pyrethrins yields of 1.9% on average.
5) P4	Released in 1970 for commercial growing in areas above 2100m above sea level. It has a flower yield of 600-800kg/ha/year and pyrethrins content of 2.0% on average. It is a hybrid from three-way cross. It's parents are 3093, 4743, and Ma/62/428. It was released in early 1970.
6) Kr/74/122	Recommended for commercial growing in areas above 2200m above sea level. It has pyrethrin content of about 2.1% and a flower yield of about 900 -1000kg/ha/year.

Ikahu. 1987; Ikahu and Ngugi. 1988.

Kabete receives about 1,046mm of rainfall per annum, which is bimodal. The long rains start in March and end in June while short rains start in October to December. It has a mean temperature of about 18° C with mean maximum and minimum temperatures of 23.4° C and 12.6° C respectively. The soils are deep dusky red to dark reddish-brown, friable clay type resistant to erosion with acid humic topsoil (Humic Nitosols) (Jaetzold and Schmidt, 1983).

Molo is located in Nakuru district in Rift Valley province of Kenya. It is on latitude 0°15' S and on longitude 35°45' E. Molo lies within the upper highland zone, at an altitude of about 2450m above sea level. It receives an average rainfall of about 1180mm per annum (Ndambuki, 1979; Jaetzold and Schmidt, 1983). The long rains start in late March to September while short rains start in October to December. It has an annual mean temperature of 13.7°C with mean maximum and minimum temperatures of 20°C and 13°C respectively. The soils are fertile, well drained, deep to very deep, dark brown, friable and smeary sandy clay with acid humic topsoil (Mollic Andosols) (Ndambuki, 1979; Jaetzold and Schmidt, 1983).

3.4 Compatibility tests

3.4.1 Preparation of clones and plant management in the field

The six parental clones were planted in April 1995 at Kabete field station in randomized complete block design. Each block was replicated three times. Each blocks had six plots of 3 x 3m. The splits were spaced 60 x 60cm to allow for the production of large, vigorous and healthy flowers. Fertilizer was applied at a rate of 6g (one teaspoonful) of double super-phosphate per hill (60-80 kg/ha of P_2O_5). The field was maintained clean by frequent weeding.

Before the onset of flowering in June 1995, the plots were covered with insect proof plastic nets with square perforations of 0.25mm^2 to keep off insects therefore preventing random cross-pollination. Each plot was covered separately by constructing a metal frame of one meter high around the plot. The plots were covered all round both on top and on the sides with the nets touching the ground. These left no space that would allow entry of insects. On flowering, flower buds were prepared for compatibility test.

3.4.2 Determination of cross-compatibility

Flower buds to be used for crossing were selected and labeled using tie-on tags. Only those that were large enough and healthy (with no signs of diseases or malformation) were selected. The labels indicated the seed parent and the pollen source for each cross. The selected and labeled flower buds were then covered by brown paper envelopes of size 10 x 7cm immersed in glycerin and dried. The envelopes were immersed in glycerin so that they do not absorb moisture or rain water that could make them heavy and fail to be supported by the flower-stalk.

Pyrethrum flowers are found in an inflorescent that have many florets. In order to ensure successful crossing, only ray florets were used for crossing as suggested by Watts (1980). The ray florets form the outermost ring and bear the white petals around the inflorescent flower. The ray florets are monosexual. They have the female parts only. Disc florets are yellow in color and occur in rings on the inside of the ray florets. The disc florets are bisexual (they have both female and male parts). All the florets do not mature and open at the same time. Openings start from the florets on the outer rings moving towards the center. The interval between one ring opening to the next is about 24 hours depending on weather. During crossing flower buds marked as female were closely monitored and

crossing was done only when the ray florets had opened. Flowers to be used as male (source of pollen grain) were uncovered and cut when a number of the disc florets had opened and shed pollen grain. They were cut in such way that the flower heads were left with a flower stalk of about 3cm to enable easy handling during crossing. Flowers cut from one parent (same clone) were put together on a petri-dish for ease of movement from one plot to the other.

During pollination, the male flower was held by the flower stalk in an inverted position above the female flower. Using a fine brush, the ray florets on the female flower were dusted with pollen grains from the inverted male flower until all the ray florets were visibly covered with pollen grains. Each clone had it's own labeled brush to avoid mix-up or contamination of pollen grain during crossing. The flowers were then covered again using brown paper envelopes of size 10 x 7cm immersed in glycerin and dried. The envelopes were then labeled indicating the date of crossing and source of pollen grains (male parent).

3.4.3 Determination of self-compatibility

Flower buds to be used for self-compatibility determination were selected, labeled and covered by brown paper envelopes of size 10 x 7cm immersed in glycerin and dried. These flower buds were closely monitored. When the disc florets started to open, pollination was carried out daily using a brush until when all the florets had opened. Each clone had it's own labeled brush. During pollination, the fine brush was gently passed around the whole flower. This was to ensure that pollen grains were disseminated from the anthers to the stigmas since the stigmas were taller than the anthers. It also ensured that pollination took place. At pollination time, the envelopes were removed and replaced

after pollination. At the end of the selfing exercise (when all the florets had been pollinated), the flowers were labeled to indicate that the flower was selfed and the date for last manual pollination.

3.4.4 Duration to seed maturity

The duration from pollination to seed maturity was determined by selecting flower heads that had exactly 2cm long dry flower stalks below the flower heads at harvesting time. This is the suitable stage for seed collection (Ikahu, 1987). The time taken from pollination to seed maturity was determined by comparing the date of harvesting to that of crossing or selfing on the envelope. Mean number of days from pollination to seed maturity was calculated for each cross and self.

3.4.5 Seed harvesting, cleaning and drying

The crossed or selfed flower heads remained covered by the envelopes until seed harvesting stage. The seeds were harvested when the disc florets had fallen off the flower heads and the flower stalk dry 2cm below the flower head. On harvesting, the flower head was picked by breaking the flower stalk about 1cm below the flower head. The harvested flower heads were put in envelopes. Seeds from crossed flower heads were removed one by one using a needle to make sure that only seed from ray-florets were harvested and not contaminated by seeds from the disc-florets. Seeds from similar crosses were put together.

Seeds from ray and disc florets from selfed flower heads were threshed manually between fingers. Seeds from each parent were bulked. Seed from the crosses and selfs were cleaned separately by removing trash manually and sorted out. The clean seeds were then

sun dried thoroughly. The seeds were spread out in open paper bags for sun drying. This prevented them from being blown away by wind.

3.4.6 Seed set

One hundred seeds were sampled out from each cross or self heads after cleaning. From these, poorly filled crinkled seeds were removed. The remaining well filled and viable seeds were counted again. Percentage pure seeds were then calculated using the following formula (Brewer, 1968).

$$\% \text{ Pure seeds} = \frac{\text{Well filled seeds} \times 100}{\text{Total number of seeds harvested}}$$

3.4.7 Seed germination test

The pure seed fraction was used for germination test. One hundred of these seeds were placed on petri-dishes with whatman's filter paper soaked in water. Each cross or self was replicated three times and kept in an incubator at 15°C (Pandita, 1983). Germination counts were done daily from day eight to day 21 when the final count was made. Percent germination was calculated after the day 21 when no more germination was observed.

$$\% \text{ Seed germination} = \frac{\text{Maximum number of germinating seeds} \times 100}{\text{Total number of seed}}$$

Percentage pure germinating seeds (PGS) were then computed using percent pure seeds and percent seed germination obtained above as shown below (Brewer, 1968).

$$\% \text{ PGS} = \frac{\% \text{ Pure seeds} \times \% \text{ Seed germination}}{100}$$

3.4.8 Pollen grains viability test

This experiment was carried out to determine the viability of pollen grain produced by six pyrethrum parental clones used in compatibility study. This was necessary since differences in pollen viability or production of none viable pollen grains can lead to wrong conclusion about compatibility reactions. This experiment was carried out at the International Livestock Research Institute (ILRI) laboratories.

Flower buds to be used were selected and covered with brown paper envelopes of size 10 x 7cm immersed in glycerin and dried. Ten flowers were covered per parent. The covered flower buds were allowed to open up. When a number of the disc florets had opened, the flowers were collected from Kabete Field Station early in the morning and taken to the laboratories. Flowers from same parent were collected on one petri-dish. In the laboratory, pollen grains from the ten flowers were collected on petri-dishes by holding the flower above the petri dish and brush-off the pollen grains. The collected pollen grains were then tested for viability using the "Hanging drop" method as described by Stanley and Linskens (1974) with a few modifications.

Pollen grains culture media was prepared by dissolving 1g of calcium phosphate ($CaPO_4$) in 100ml of distilled water. The $CaPO_4$ was used as a buffer to maintain the pH at 6.8. From the solution made, 60g was weighed and put in a beaker. Forty grams of pure sucrose powder was added to make 100g of 40% sucrose solution. The sucrose acts as a source of nutrients to the germinating pollen grains. 0.055g of boric acid was added to 99.945g of the sucrose solution to make 100g of a solution with 0.01% boron as boric acid. Boron was found to be necessary for normal growth of pollen tubes (Stanley and Linskens, 1974). 0.3g of polyoxyethelen was added to the 100g of the solution. The role

of polyoxyethylene was to break the surface tension of the solution so that the pollen grains can sink in the culture media and also to act as a dispersing agent so as to separate pollen grains that might be glued together in to single separate pollen grains.

Eighteen microscope slides were prepared three for each clone. Using petroleum jelly (Vaseline), three small circles were made on each slide. A drop of the culture media was placed in the center of each circle. The petroleum jelly circle helped in holding the drop so that it does not spread on the slide. On these culture media drops, 10 to 20 pollen grains were dusted. Pollen grains from each parent were dusted on three slides each with three drops. The slides were then rested on wooden rods in petri dishes containing wet absorbent paper. The slides were rested in an inverted position in order to allow pollen grains to be at the base of the drops so that the developing pollen tube would grow within the media ensuring supply of nutrients since pollen tubes exhibit geotropism. The preparations were kept in an oven at temperatures of 30°C.

Data was collected by observing these slides under a microscope. During observation, total number of pollen grains per drop per slide was recorded. Also pollen grains germinating (whose exine had broken) per slide were counted. Observations were made after 24, 48, 72 and 96 hours. From these, average percentage pollen grains germination per parent was calculated as follows.

$$\%\text{Pollen grain germination} = \frac{\text{Total number of germinating pollen grains per slide} \times 100}{\text{Total number of pollen grains on the slide.}}$$

$$\text{Average \% pollen grain germination} = \frac{\sum \text{ of \% pollen grain germination per slide}}{\text{Total number of slides observed}}$$

3.4.9 Pollen tube growth

This experiment was carried out as a follow up to confirm the observations made in the field on compatibility test.

3.4.9.1 Preliminary trial

To our knowledge use of fluorescence in observing pollen tube growth in pyrethrum has not been reported anywhere. Therefore preliminary study was conducted to determine the best concentration level of sodium hydroxide (NaOH) and duration of treatment to be used as described by Kho and Baer (1968). Two samples were used in this experiment. Pollen grains from P4 and crossed flowers from clone P4 x L/64/43331.

Crossing was carried out by covering the flower buds of the parents with brown paper envelopes of size 10 x 7cm immersed in glycerin and dried. Pollination was precisely done when only the ray florets of the female flower had opened out. The pollinated flowers were picked early in the morning from Kabete field station on the third day after pollination and taken to the laboratory. In the laboratory, the styles from the ray-florets were carefully removed under a dissecting microscope. They were then treated with sodium hydroxide at five different concentrations (0.5, 1, 2, 5 and 10N). Each concentration was replicated four times. The treatments (petri dishes with NaOH) and the styles were kept in an oven at 30°C. This gave enough time and conditions for the NaOH to soften and decolorize the stylar tissues.

One petri dish at each concentration was removed after ½, 1, 2 and 6 hours for further treatment and observation. On removal from the oven, the styles were washed thoroughly in distilled water, dried using absorbent paper before treatment in a solution of 0.1%

aniline (water-soluble) dissolved in 0.1N potassium phosphate (K_3PO_4) for about one hour. The styles were then placed in a drop of 0.1% aniline in 0.1N K_3PO_4 or glycerin on a slide to prevent rapid drying out. The styles were then quashed under coverslips carefully to avoid scattering off the tissue parts. The slides were then observed under a fluorescent microscope using Osram HBO 200W high pressure Mercury (Hg) lamp as source of light. Observations were made immediately to avoid loosing the intensity of florescence after aniline treatment. After observing all the slides, it was concluded that a treatment with 2N NaOH for ½ to 1 hour of the styles gave the best results. At these levels of treatment, the pollen tubes could easily be seen and distinguished from the stylar tissue.

3.4.9.2 Time course study

Compatibility (both cross and self) are determined by the ability of viable pollen grains to stick and germinate on the stigmas, develop pollen tubes, grow down the style and finally to cause fertilization by fusing with the egg in the ovary. Failures of any of these steps in this process to take place leads to no fertilization and presumed incompatibility. When there is deliberate rejection of pollen (incompatibility) there is often callose deposition to block growth of the incompatible pollen grains in the stylar transmitting cells and this can be observed microscopically. By following events after pollination, it is possible to determine whether two crossed plants are compatible or not. It is on this basis that this experiment was carried out as a follow up to confirm what had been observed in the field. Three known cross compatible clones, P4, Sb/66/107 and L/64/4331 two suspected cross incompatible clones Sb/66/107 and Ma/75/4 and selfed flowers from clone P4 and L/64/4331 were used in this study.

Flower buds to be used for crossing were covered by small paper envelopes of size 10 x 7cm immersed in glycerin and dried. Ten flowers were crossed in each set (10 each from compatible clones, incompatible clones and selfs). For cross compatible clones, clones L/64/4331 and Sb/66/107 were used as male (source of pollen) parent while clone P4 was used as female parent. For suspected cross incompatible clones, clone Sb/66/107 was used as female parent while Ma/75/4 was used as male parent. During pollination flower buds marked as female were closely monitored and crossing was done only when the ray florets had opened. Flowers to be used as male (source of pollen grain) were uncovered and cut when a number of the disc florets had opened and shed pollen grain. They were cut in such way that the flower heads were left with a flower stalk of about 3cm to enable easy handling during pollination.

During pollination, the male flower was held by the flower stalk in an inverted position above the female flower. Using a brush, the ray florets on the female flower were dusted with pollen grains from the inverted male flower until all the ray florets were visibly covered with pollen grains. Each clone had it's own labeled brush to avoid mix-up or contamination of pollen grain during crossing. The flowers were then covered again using brown paper envelopes of size 10 x 7cm immersed glycerin and dried. The envelopes were then labeled indicating the date of crossing and source of pollen grains (male parent).

Selfing followed a similar procedure as that used in crossing outlined above only that the flower heads used as source of pollen (male parent) were from the same clone as female. Only the ray florets were used during selfing because precise time from pollination to

pollen tube observation was necessary in this study. Crossing and selfing was done at Kabete field station.

The crossed and selfed flowers were picked daily from the field early in the morning. One flower from each set was collected daily and taken to the laboratory for seven days. The styles from the ray florets were carefully removed under a dissecting microscope. The styles were then treated in 2N sodium hydroxide (NaOH) and kept in an oven at 30°C for ½ to 1 hour. The styles were then washed thoroughly in distilled water and dried using absorbent paper. They were then treated in a solution of 0.1% aniline (water-soluble) dissolved in 0.1N K_3PO_4 for about one hour. The material was then placed on microscope slides containing glycerin or 0.1% aniline in 0.1N K_3PO_4 to prevent rapid drying out. The material was then squashed under a coverslip carefully to avoid scattering off the tissue parts. The slides were then observed under fluorescent microscope using Osram HBO 200W high-pressure mercury (Hg) lamp as source of light. Observations were made immediately to avoid loosing the intensity of florescence after aniline treatment. Data was taken by scoring approximate level of development of the pollen on the stigmas and length of the pollen tubes in the styles. These lead to formulation of a rating scale procedure shown.

Index	Description
0	No germination no pollen tubes observed
1	There is germination and pollen tubes less than 1/4 the style length.
2	Pollen tube between 1/4- 1/2 the style length.
3	Pollen tube over 1/2 but not reaching the base of the style.
4	Pollen tube grown past the base of the style.

3.5 Heterosis and combining ability analysis

3.5.1 Seedling development in the nursery

The seeds of F_1 and their reciprocals for a complete 6 x 6 diallel were obtained as described in Experiment 1. The seeds were sown in the nursery at Kabete field station in February 1996. F_1 and reciprocal seeds from each set of parents were sown in a separate nursery of 1 x 1m in size. The seeds were sown in shallow rows of about 1 to 2cm deep. The spacing between the rows was 10cm. The rows were then covered using a thin layer of dry grass mulch after sowing. The seedlings in the nursery were then watered daily. On emergence, the dry grass mulch was removed and a shade was constructed above the nursery. The shade was constructed using metal frames at a height of about 1m high. Dry grass covers was put on top of a metal framework to provided enough shade to the emerging seedlings during harsh weather conditions and also to reduce the velocity of raindrops that could otherwise injure or break the seedlings. The intensity of the shade kept on being reduced as the seedlings developed by reducing the thickness of the grass cover thus hardening the seedlings. Thinning was done in cases of overcrowding.

In May 1996, when the seedlings were between 8cm to 10cm tall, they were then transplanted in 'expansion field'. This was necessary since we required large plants that could be split and planted in the two experimental sites. In the 'expansion fields', the seedlings were planted at a wider spacing of 60 x 60cm. Fertilizer was applied at a rate of one teaspoonful (6g) per hill (60-80kg/ha of P_2O_5). In the expansion field seedlings from a single cross were planted in a single row. The plots were kept clean by frequent weeding and irrigated if need arose. In September 1996, the seedlings were large enough to produce enough splits to be planted in the experimental plots.

3.5.2 Field layout and management

The experiments were planted during short rains in October 1996 at Kabete and during long rains in April 1997 at Molo. Splits from the parents, F_1 and reciprocals were grown in the two locations in order to evaluate their performance. The experimental design was a randomized complete block with three replications. Each replicate had 15 plots. A plot had 12 rows, each 1.5m long. The two middle rows consisted of the two parents of the cross while the other 5 rows on either side consisted of the F_1s on one side and the reciprocals on the other side. Between row spacing was 60cm while within row spacing was 30cm. A 1m wide path was left in-between the plots to allow easy working within the plots. Two rows of clone 4331 were planted around the blocks as guard rows. Fertilizer was applied at a rate of one teaspoonful (6g) per hill (160-180kg/ha of P_2O_5) of double super phosphate and thoroughly mixed with the soil to avoid direct contact with plant roots before planting. Other routine field maintenance practices like weeding and earthing up were carried out.

3.5.3 Data collection

The following data was collected:

1) Bush diameter (cm): Three middle plants per row were used in taking this parameter. Bush diameter was taken by measuring the cross-section of the plant at the base. (Pyrethrum Board of Kenya Annual report. 1993). This was taken every month in both locations and the averages for each plot from F_1 hybrids, their reciprocals and parents calculated.

2) Plant height (cm): Plant height was recorded in three middle plants per row using a meter ruler. The meter ruler was placed straight in the middle of the plant touching the ground (base of the plant) and a reading for the tallest stem on the plant taken. This was taken every month in both locations and the averages for each plot from F_1 hybrids, their reciprocals and parents calculated.

3) Flower yields (kg/plot): Mature flowers were picked by hand every two weeks from each plot and weighed using an electronic balance at Kabete field station and National Pyrethrum and Horticultural Research Center, Molo. From these, fresh flower weight were obtained and recorded per plot from the F_1 hybrids, reciprocal and their parents per harvest. After obtaining the fresh flower weight from each plot, the fresh flowers were then put in labeled paper bags and dried in an oven at 80°C for about 2 hours and then at 50°C for another 2 hours. The samples were air cooled, weighed and put again in the oven at 100°C for 3 hours and left overnight at room temperature, then weighed again and kept at room temperature and re-weighed after 2 days. The average weight of the last two readings was taken as the dry flower weight per plot for the F_1 hybrids, reciprocal and their parents. These dry flower weights par plot was used to determine flower yields (Kg/ha) by multiplying by 10,000 and dividing by the plot size.

4) Percent dry flower weight (%): This was taken as a percentage of the dry flower weight over the fresh flower weight per plot for the F_1 hybrids, reciprocal and their parents.

5) Pyrethrins content (%): The pyrethrins content was determined using the Beckley's u.v. spectrophotometric method (Beckley, 1950) . The analysis was carried out at the

Pyrethrum Board Chemistry laboratory, Nakuru. The analysis was done per plot of F_1 hybrids, their reciprocals and parents.

6) Pyrethrins yield (kg/ha): This is a product of dry flower yields (kg/ha) and pyrethrins contents (%). Pyrethrins yields (kg/ha) = Dry flower yield (kg/ha) x % pyrethrins content Mean values for the parents and their F_1 hybrids were then computed. Analysis of variance was carried out to determine genotypic differences if there were among the parents alone and the parents plus the F_1 hybrids for each trait in each location separately.

3.5.4 Data analysis

3.5.4.1 Heterosis

Heterosis for plant height, bush diameter, flower yield, pyrethrins contents and pyrethrins yields were expressed as percent F_1 heterosis over the better or high parent value (Allard, 1999: Suresh and Renu, 1975). The following formula was used:

$$H\ (\%) = \frac{(F_1 - HP) \times 100}{HP}$$

Where: H = Heterosis (%)

F_1 = Hybrid mean value

HP = High parent value

3.5.4.2 Diallel analysis

Data from parental, F_1s and reciprocal were first subjected to analysis of variance to test the significance of genotypic differences. In this analysis, mean value of the parent, F_1s and reciprocal per plot per replication for each character per location were used. Mean

separation was carried out using least significant differences (LSD) (Steel and Torrie, 1980) for the purpose of comparing performance at Kabete and Molo. This was done only when genotypic difference were detected according to the formula:

$$LSD = t_{\alpha/2}\sqrt{\frac{EMS}{r}}$$

Where: -LSD = Least significant difference

EMS = error mean square

r = replicates

$t_{\alpha/2}$ = tabulated t value for an α level test against two sided alternatives using error degrees of freedom.

After analysis of variance, the data from parents, F_1s and reciprocal were analyzed according to Griffing's [1956(a)] combining ability analysis using Method 1 (parents, F_1s and reciprocals) and Model 1. For combining ability analysis, the data was rearranged into replication mean values.

The statistical model for combining ability analysis in Method 1, Model 1 is:

$$Y_{ij} = M + g_i + g_j + S_{ij} + r_{ij} + 1/bc \sum\sum eijkl$$

i,j = 1,2,3,_____,n

k = 1,2,3,_____,b

l = 1,2,3,_____,c

Where: - Y_{ij} = the mean of i x j^{th} genotype over k and l

g_i = the general combining ability (gca) effect of its parent

g_j = the gca effect of j^{th} parent

s_{ij} = the interaction specific combining ability (sca) effects for the cross between the i^{th} and j^{th} parent

r_{ij} = the reciprocal

$1/bc \sum\sum eijkl$ = the mean error effect .

K and l = different environmental conditions (locations)

The combining ability effects were tested by comparing with a standard/critical value (sv) calculated as follows:

$$SV = SE \times t = \sqrt{[\text{variance} \times t_{(\text{tabulated})}]}$$

Where; - SE = standard error.

Each comparison was a two-tailed test with error degrees of freedom. All calculations followed a worked example by Sigh and Chaudhary (1977).

CHAPTER 4

RESULTS

4.1 Effects of environmental temperatures on flower opening and pollen grains shedding and pollination

During early morning and on the days when day's maximum temperatures were below 20°C, the flowers tagged as female and expected to open failed to do so. These flowers remained closed until when the temperatures rose to above 20°C. Similarly, flowers tagged as male failed to shed pollen grain or the pollen grains shed under this condition were glued together. These made it hard to carry out pollination when the temperatures were below 20°C (Table 2). When maximum daily temperatures were below 20°C, the number of crosses carried out was low. There was high correlation between daily maximum temperatures and number of crosses (r = o.68*).

On the 1, 5, 11, 18, 22 25, 26, and 27 July, 1995 when the day's maximum temperatures were below 20°C, the number of successful crosses done was less than ten (Tables 2). The lowest and maximum number of successful crosses was 5 and 28 respectively. These crosses were done on 26 and 24 July, 1995 when maximum temperatures were 18.7° C and 21.2° C, respectively. The coldest day was on the 27 July, 1995 when minimum and maximum temperatures were 10.9°C and 17.9°C. Seven successful crosses were made on this day. The mean number of crosses done was 17.5 per day while mean minimum and maximum daily temperatures for the month were 11.4° C and 21.1° C, respectively (Table 2).

Table 2: Daily temperatures and number of crosses done per day in 6 pyrethrum clones.

Date	Min. temperature (°C)	Max Temperature (°C)	No of successful crosses
1/7/95	10 6	18 8	8
2/7/95	11.1	20.1	22
3/7/95	11.0	21 3	7
4/7/95	10 8	21.6	26
5/7/95	11.2	19 7	9
6/7/95	11.1	20 7	19
7/7/95	11.9	20.9	27
8/7/95	12.0	22 6	12
9/7/95	11.7	21.8	23
10/7/95	10.0	20.6	21
11/7/95	11.1	19 8	7
12/7/95	12.5	21.7	19
13/7/95	11.5	22.8	22
14/7/95	11.4	21.9	21
15/7/95	12.6	23.4	26
16/7/95	13.3	24.0	23
17/7/95	14.1	20.4	26
18/7/95	11.2	17.9	7
19/7/95	12.9	23 6	31
20/7/95	11.2	21.7	22
21/7/95	11.8	20.4	17
22/7/95	10.3	19.9	8
23/7/95	11.7	22.3	21
24/7/95	10.6	20.2	28
25/7/95	10.8	19.8	6
26/7/95	10.9	18.7	5
27/7/95	10.9	17.9	7
28/7/95	10.8	20 0	10
29/7/95	11.0	22.5	19
30/7/95	11.2	22.7	23
31/7/95	12.7	24 0	20
Mean	11.4	21.1	17.5

Correlation coefficient between maximum temperatures and number of pollination was $r = 0.678^{**}$

4.2 Success in crossing and selfing

Among the six parental clones used in crossing and selfing. the average number of ray florets ranged from 14 to 22 with mean number of 18.2 per flower head (Table 3). Clone L/64/4331 had the highest (22) while clone P4 had the lowest number of ray floret (14). The total number of florets (both disc and ray florets) ranged between 118 to 188 with a mean of 162.5. Clone Ks/71/6 had the highest total number of florets (188) while clone P4 had the lowest number (Table 3). Pure seeds were well-filled and bright brown in color while impure seed were dark brown in color and wrinkled (Plate 1).

4.3 Duration from pollination to seed maturity

Hybrids from cross P4 x Ks/70/64 took the longest duration from crossing (pollination) to seed harvesting (54 days) while cross Ks/70/64 x L/64/4331 took the shortest duration of and 47.7 days. (Table 4). The mean number of days from pollination to seed harvesting was 50.7 days. There were no significant differences in number of days among the crosses and the reciprocals ($P \geq 0.05$) (Table 4).

4.4 Success rate, seed set and quality

Hybrid seeds from the six parents had percentage pure seeds ranging from 34.9% to 89.4% with a mean of 62.7% (Table 5). Crosses Ks/70/64 x Ks/71/6 and P4 x Ks/70/64 had the highest and lowest percentage pure seeds of 89.4% and 34.9%. respectively (Table 5). Percentage seed germination ranged between 10% and 60% for crosses L/64/4331 x Ks/74/122 and L/64/4331 x Ks/70/64. Mean percentage seed germination was 31.3% (Table 5).

Table 3: Average number of ray florets and disc florets per flower head of six pyrethrum clones grown at Kabete.

Parent	Ray florets	Disc florets	Total number of florets
Sb/66/107	18	145	163
P4	14	104	118
Ks/74/122	18	153	171
Ks/71/6	17	171	188
L/64/4331	22	151	173
Ks/70/64	20	142	162
Mean	18.2	144.3	162.5

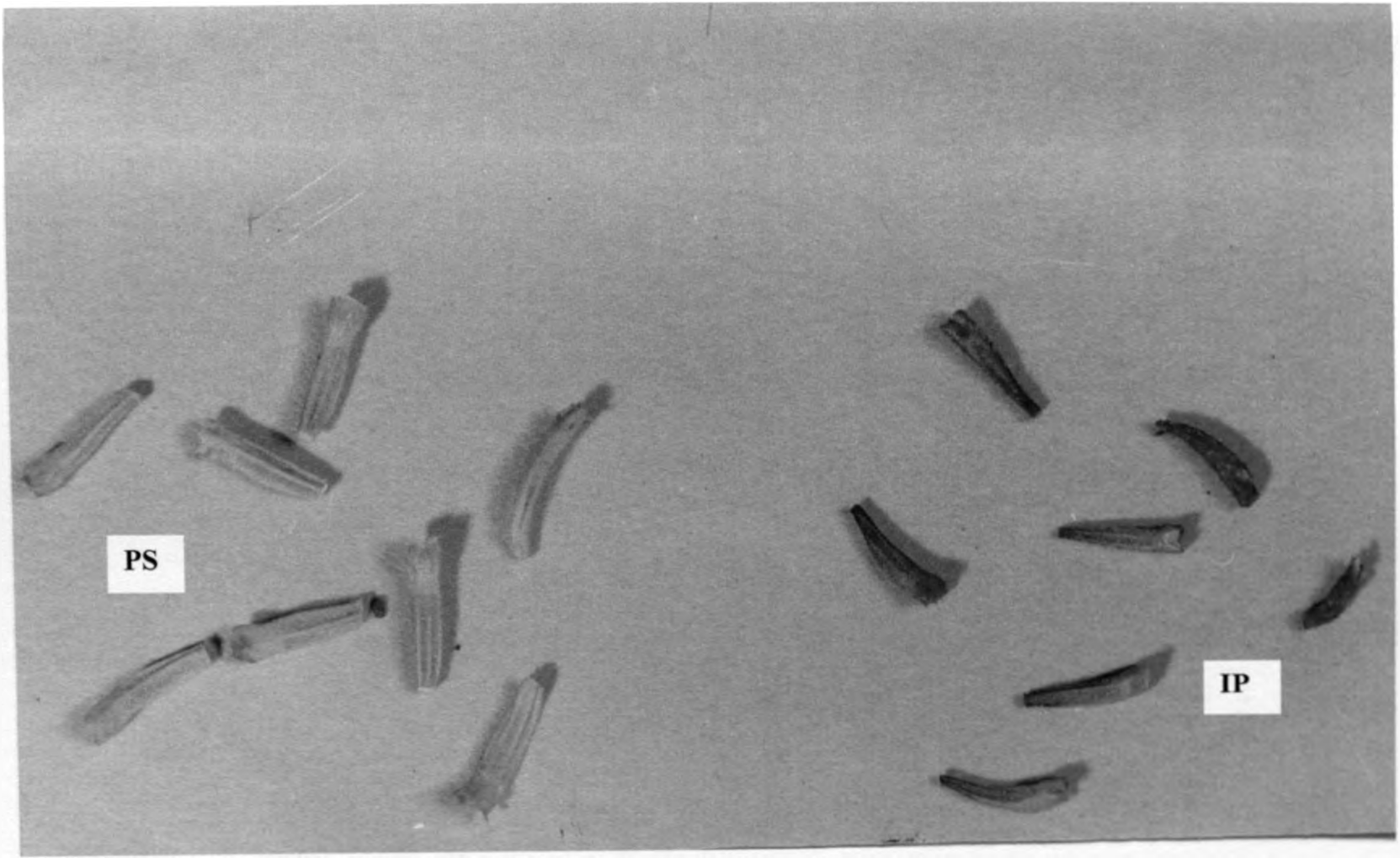


Plate 1: Pure (ps) and impure seeds (Ip) of Pyrethrum from clone P4

Table 4: Number of days from pollination to seed maturity in selfs from six pyrethrum clones and 15 crosses and their reciprocals.

Parent/Cross	Mean number of days to maturity	
	Cross pollination	Reciprocal cross
Sb/66/107 x P4	50 3	51 0
Sb/66/107 x Ks/74/122	49 7	50 3
Sb/66/107 x Ks/71/6	51.3	51 0
Sb/66/107 x L/64/4331	53 3	51.3
Sb/66/107 x Ks/70/64	51.7	52.0
P4 x Ks/74/122	51.0	49 3
P4 x Ks/71/6	52.0	50 7
P4 x L/64/4331	51.7	52.0
P4 x Ks/70/64	54 0	50.7
Ks/74/122 x Ks/71/6	50.7	52.7
Ks/74/122 x L/64/4331	47.7	48 3
Ks/74/122 x Ks/70/64	48.7	49.0
Ks/71/6 x L/64/4331	51.7	51.3
Ks/71/6 x Ks/70/64	51.3	50.0
L/64/43331 x Ks/70/64	50.0	47.7
Mean	51.7	50.5
$LSD_{(0.05)}$	8.7	9.0
Overall mean	50.7	

Table 5: Percent pure seeds, seed germination and pure germinating seeds of 15 pyrethrum F_1 hybrids and their reciprocals.

Cross	% Pure seeds		% Seed germination		%P G S	
	F_1	Recip	F_1	Recip	F_1	Recip
Sb/66/107 x P4	70.2	61.1	60 0	48 0	42 1	29 3
Sb/66/107 x Ks/74/122	53 7	79 7	30.0	36 0	16 1	28 7
Sb/66/107 x Ks/71/6	66.7	62.8	32 0	12 0	21.3	7.5
Sb/66/107 x L/64/4331	56 8	72.1	18 0	26 0	10.2	18 8
Sb/66/107 x Ks/70/64	62.3	63 2	30.0	30.0	18 7	19 0
P4 x Ks/74/122	58.1	74.2	24.0	32.0	13.9	23.7
P4 x Ks/71/6	49.7	61.7	46.0	20.0	22 9	12.3
P4 x L/64/4331	42.8	47.5	40.0	14.0	17.1	6.7
P4 x Ks/70/64	34.9	42.1	24.0	18 0	8.4	7.6
Ks/74/122 x Ks/71/6	84.7	67.8	54 0	32.0	45 7	23.1
Ks/74/122 x L/64/4331	61.0	57 4	36.0	10 0	22.0	5.7
Ks/74/122 x Ks/70/64	64.6	62.1	30.0	36 0	19.4	23.4
Ks/71/6 x L/64/4331	52.6	59.3	46.0	52.0	24.2	30 8
Ks/71/6 x Ks/70/64	68 7	89.4	44.0	30.0	30.2	26 8
L/64/4331 x ks/70/64	70.2	77.6	10.0	18.0	7.0	14.0
Mean	59.8	65.6	34 9	27 7	21.3	18 4
$LSD_{(0\ 05)}$	13.9	13.7	14.5	14.4	8.9	8.5

% PGS = Percent pure germinating seeds, Recip. = Reciprocal cross.

F_1 from Ks/74/122 x Ks/71/6 gave the best seed set of 45.7% pure germinating seeds while cross L/64/122 x Ks/74/122 had the lowest percentage of 5.7% (Table 5). The mean percent pure germinating seeds for F_1s, reciprocal and overall were 21.29%, 18.42% and 19.86% respectively (Table 5). There were no significant differences ($P \geq 0.05$) percentage pure seeds, seed germination and germinating seeds between F_1 crosses and their reciprocals. However, there were significant differences among the crosses at both 0.05 and 0.01 probability levels in both the F_1 crosses and the reciprocals

All the seeds from the selfs were not viable. The seeds were wrinkled and dark in color. They all failed to germinate thus percentage pure seeds and percent seed germination was zero.

Percent pure germinating seeds ranged from 5.7% to 45.7% (Table 5). This implies that from a single cross (crossing one flower head whose ray florets ranged from 14 to 22) the expected number of seeds ranges from (14 x 5.74/100 = 0.804) which is approximately one seed to (22 x 45.75/100 = 10.063) which is approximately ten seeds. This range can be used as a measure of success of crossing in a crossing program. In this case, pollination per flower head (capitulum) would yield between one to ten germinating seeds

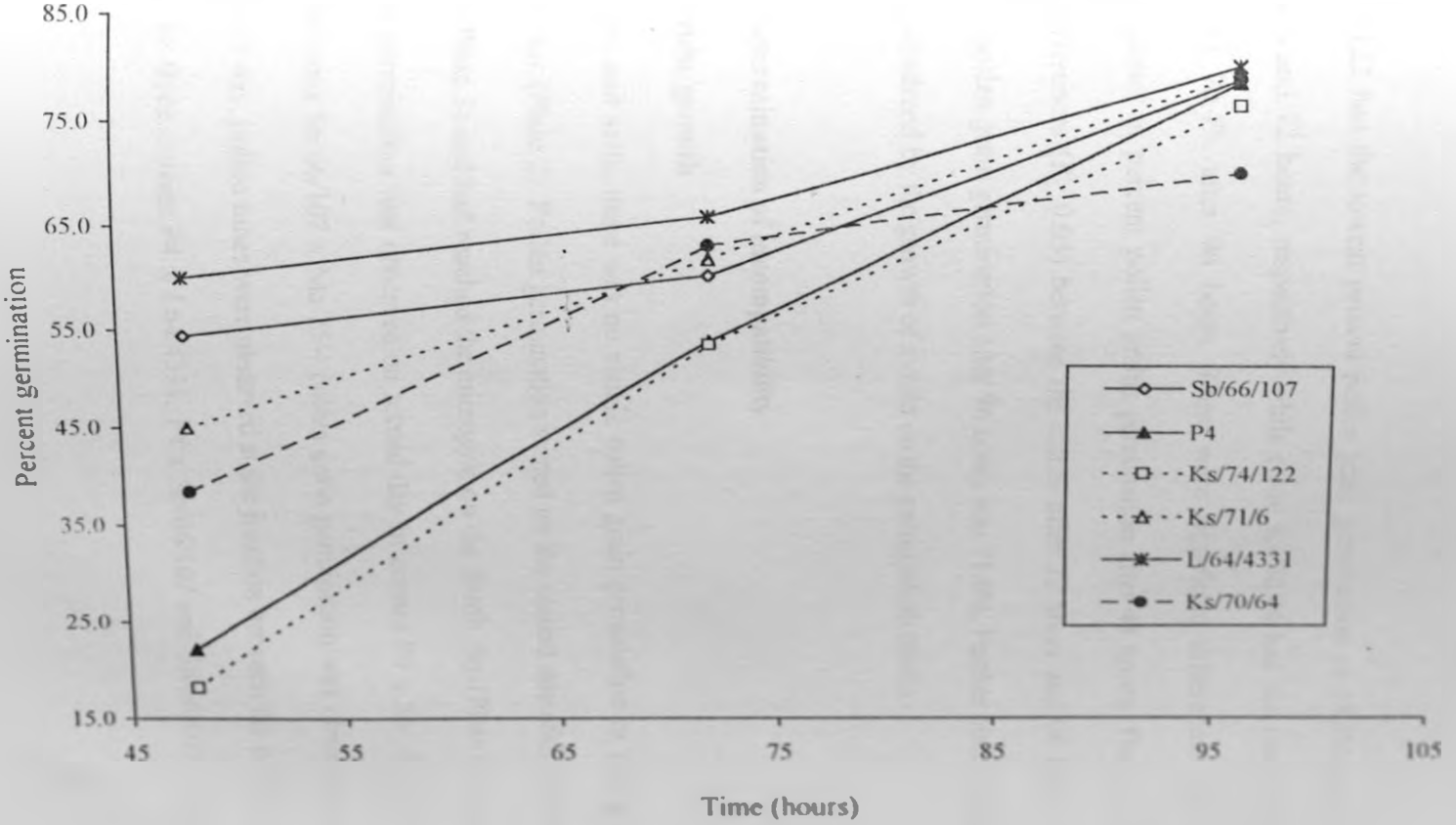
4.5 Pollen grains viability

Among the six parental clones tested, clone L/64/4331 had the highest pollen grain germination percentage of 80.4% after 96 hours (Table 6). Clones, Ks/70/64 expressed the lowest percentage of 70.4% after 96 hours (Table 6, Figure 3).

Table 6: Percent pollen grains germination on sucrose growth media of six pyrethrum clones after 48,72 and 96 hours.

Parent	Percentage germination		
	48 hours	72hours	96hours
Sb/66/107	54 4	60.5	79 2
P4	22 2	54 0	78 9
Ks/74/122	18.3	53.7	76.7
Ks/71/6	45 0	62.1	79.8
L/64/4331	60.1	66 1	80.4
Ks/70/64	38.3	63.4	70.4
Mean	39.7	60.0	77.6
$LSD_{(0\ 05)}$	11.5	11.0	7.8

Figure 3. Percent germinating pollen grains on sucrose media for six pyrethrum clones over time (hrs).



In all the clones, there was a steady increase in percentage germination of pollen grains with time. Clone L/64/4331 showed the highest pollen grains germination percentage of 60.1% after 48 hours and maintained the leading position up to 96 hours.

Clone Ks/74/122 had the lowest percent pollen grain germination of 18.3% and 53.7% after 48 hours and 72 hours, respectively while clone Ks/70/64 had the lowest percent germination of 70.4% after 96 hours. There were significant differences ($P \geq 0.05$) between the clones in percent pollen grains germination after 48 hours. There were no significant differences ($P \geq 0.05$) between the clones after 72 hours and 96 hours. The mean percent pollen grain germination after 96 hours was 71.6%. Further observation and counting was hindered by the growth of mould on the germination media.

4.6 *In-vitro* determination of incompatibility

4.6.1 Pollen tube growth

In both crosses and selfs, there was no visible pollen grain germination or pollen tubes after the first day (Plate 2). Pollen germination started on the second day after pollination (Table 7 and Plate 3) and had reached the micropyle on the fourth day (Plate 4). The first pollen grains germination was observed on second day in crosses P4 x Sb/107 and P4 x L/64/4331. In cross Sb/66/107 x Ma/75/4 pollen grain germination was observed on day 3. On the fourth day, pollen tubes were observed at the junction between the pistil and the ovary in all the three crosses P4 x L64/4331, P4 x Sb/66/107 and Sb/66/107 x Ma/75/4 (Plate 4).

Table 7: Daily index for the length of pollen tubes in three crosses and two selfs of pyrethrum.

Cross	Days after pollination						
	1	2	3	4	5	6	7
P4 x Sb/66/107	0	1	4	4	4	4	4
P4 x L/64/4331	0	1	3	4	4	4	4
Sb/66/107 x Ma/75/4	0	0	4	4	4	4	4
P4 x P4	0	0	0	0	0	0	0
Sb/66/107 x Sb/66/107	0	0	0	0	0	0	0

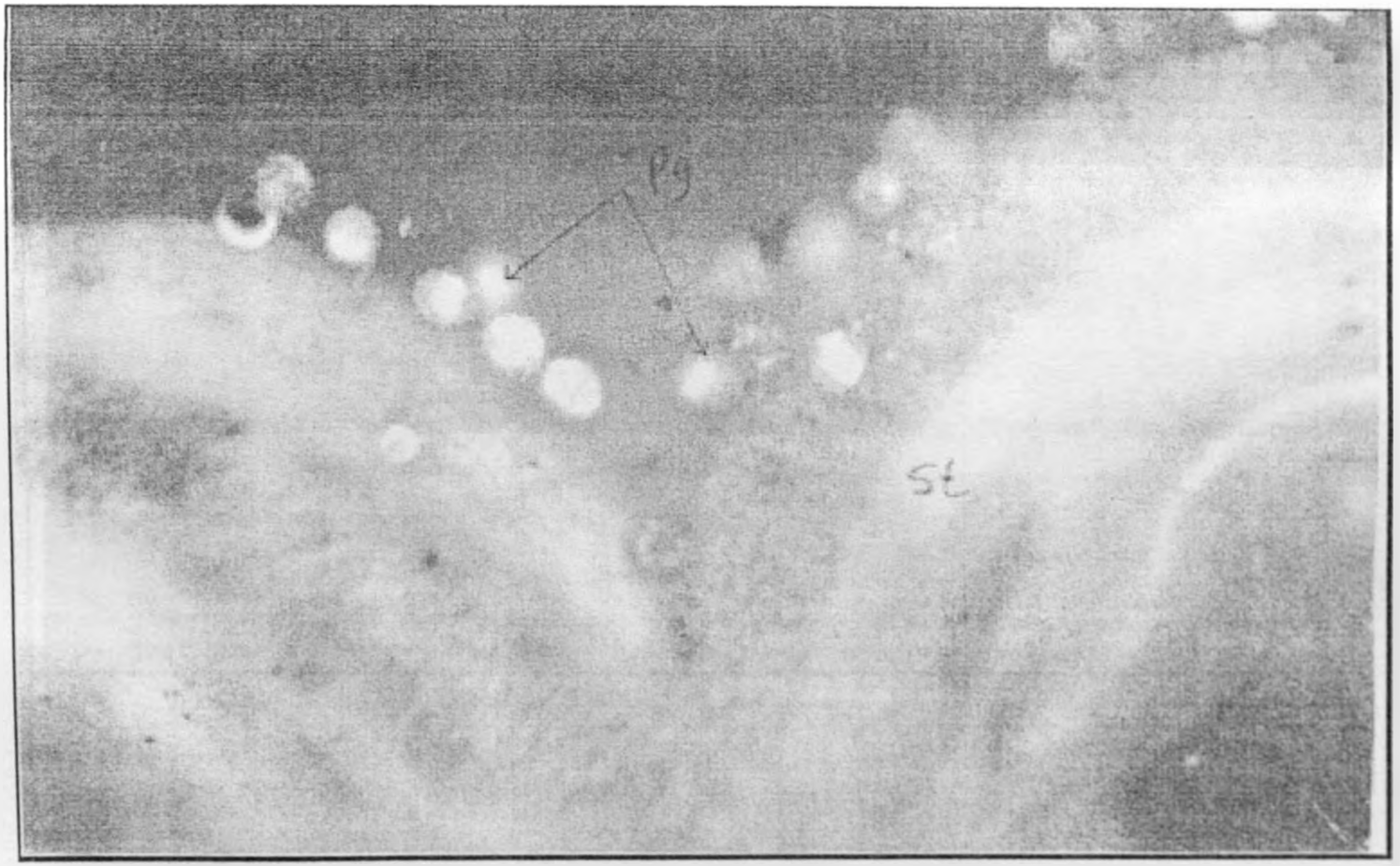


Plate 2: Pollen grains (pg) on the stigma (st) of clone P4 on day 1 after pollination

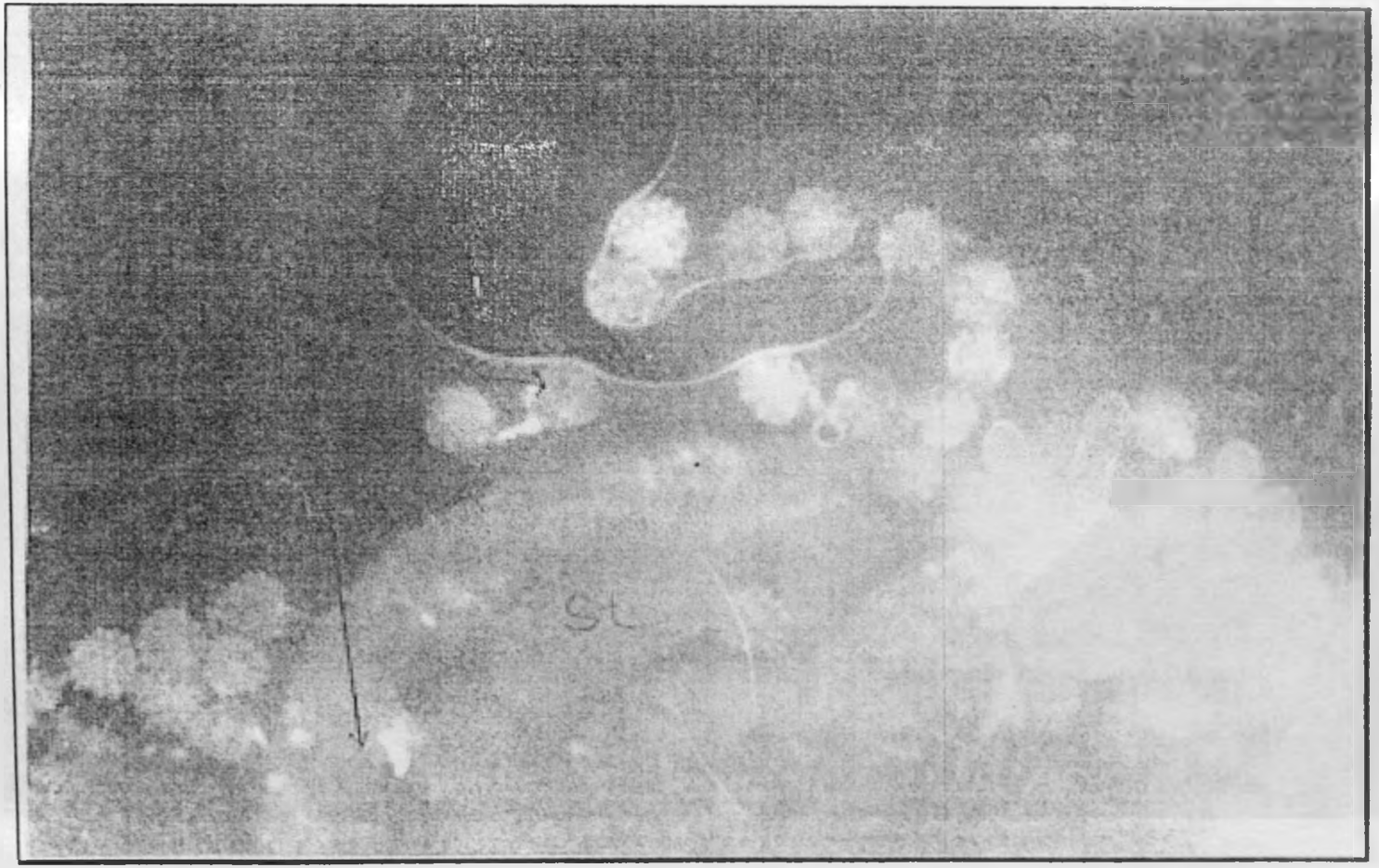


Plate 3: Germinating pollen grains (pg) on the stigma (st) of clone P4 0n day 2 after crossing with I/64/4331.

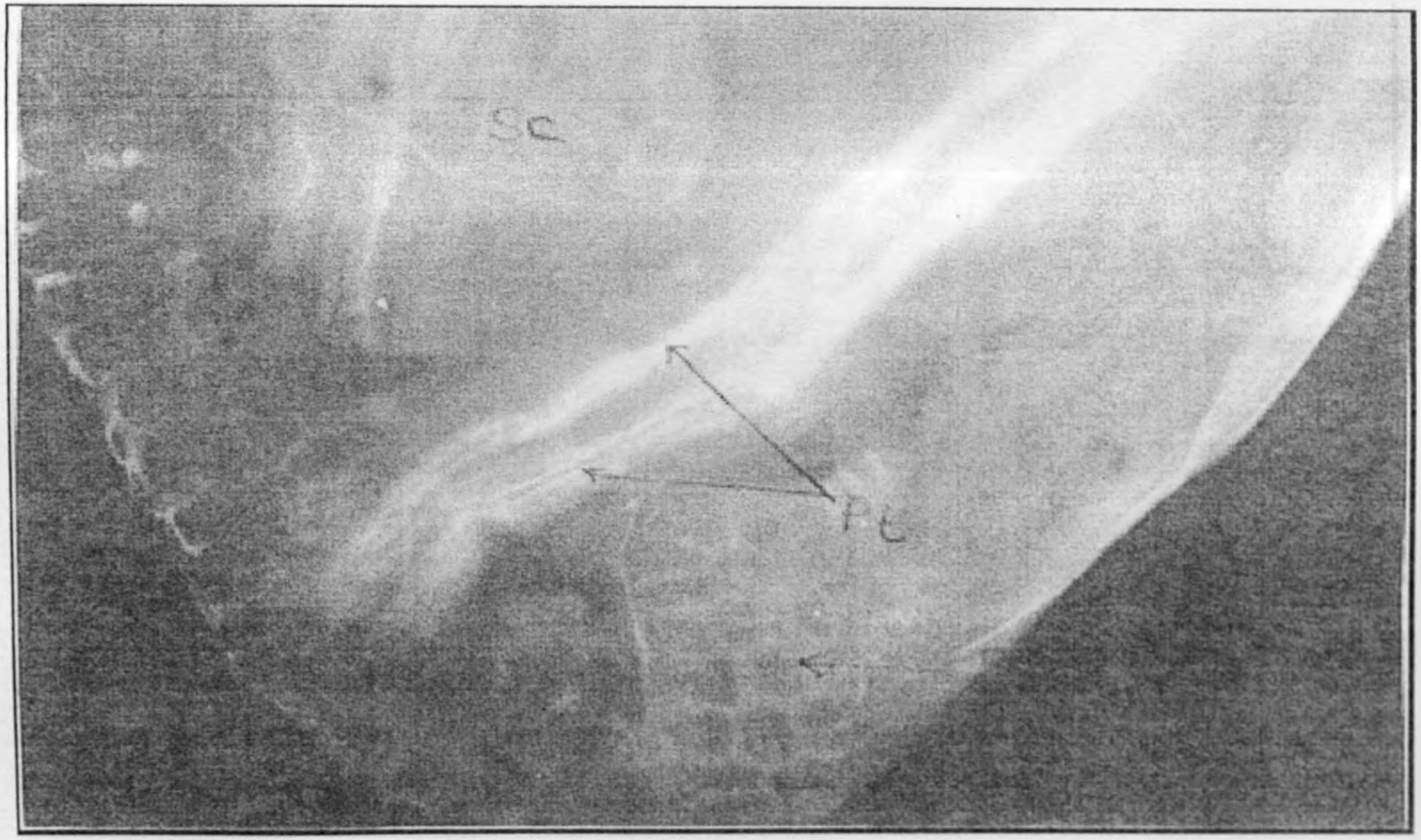


Plate 4: Pollen tube (pt) at the base of the stylar tissue in clone P4 on day 5 after crossing with L/64/4331. The cells at the stylar-ovary junction (sc) can clearly be seen,

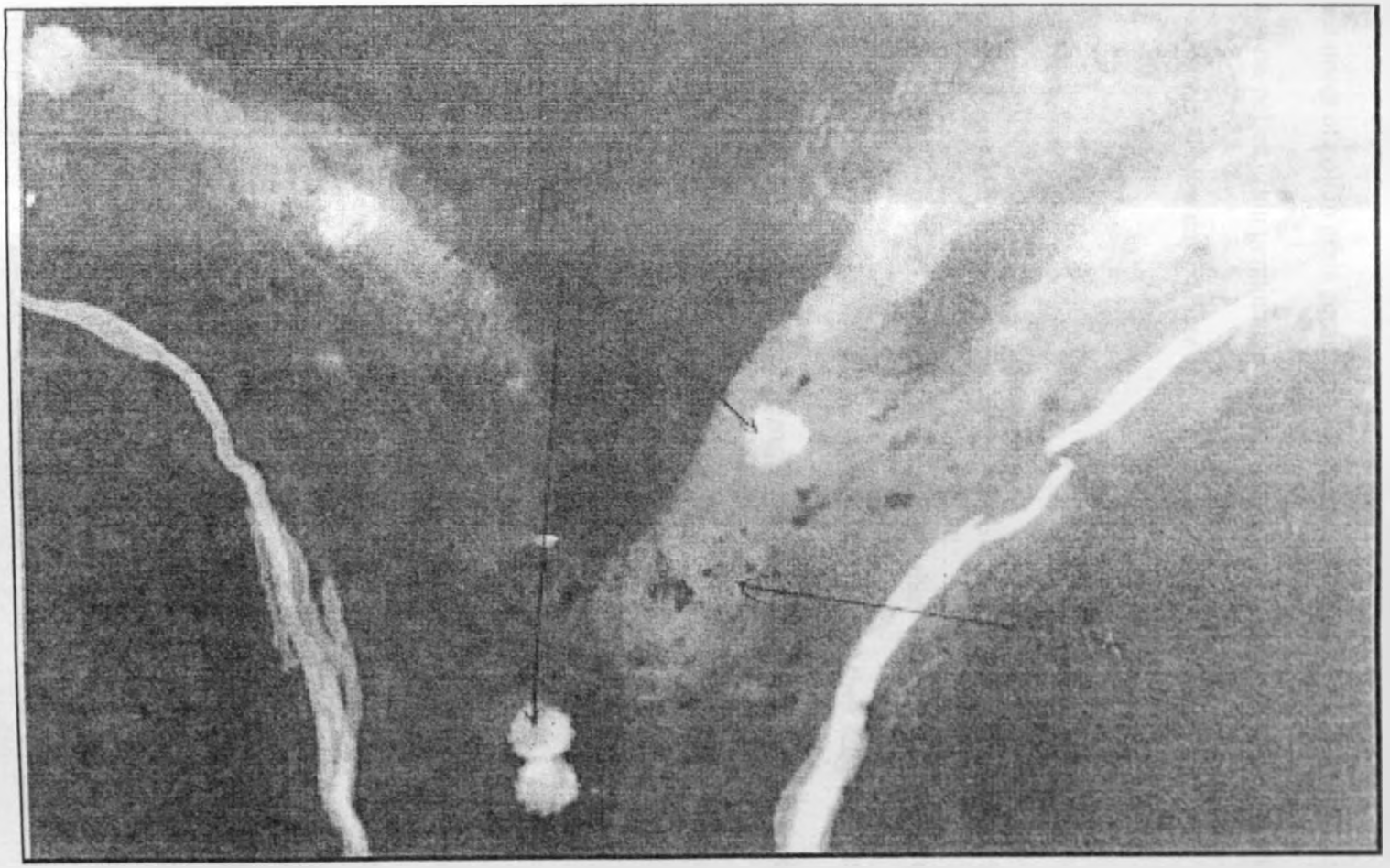


Plate 5: Self pollen grains (pg) failed to germinate on the withered stigma (st) of clone P4 on day 7 after selfing.

Pollen tubes in the ovary could not be observed because the ovaries got disjointed from the pistils during treatment. Crosses P4 x L64/4331 and P4 x Sb/66/107 had a relatively fast onset of pollen grain germination than cross Sb/66/107 x Ma/75/4. In the selfs, there was no pollen grain germination all through from day 1 to day 7, their styles turned brown and withered after day 6 (Plate 5).

4.7 Heterosis

Hybrid plants and their reciprocals from a 6 x 6 complete diallel crosses were analyzed for heterosis. Table 8 presents mean performance values of six pyrethrum parental clones. Table 9 shows mean performance values of F_1 hybrids and their reciprocals at Kabete and Molo. Table 10 shows percent heterosis of F_1 hybrids over their high parent values at Kabete and Molo.

4.7.1 Bush Diameter

Hybrid P4 x L/64/4331 when grown at Kabete and L/64/4331 x Ks/74/122 at Molo had the widest bush (Table 9). Bush diameter ranged from 30.1 cm to 50.5 cm. The hybrids were wider at Kabete than at Molo. Significant differences in bush diameter among the parents were detected at Kabete but not at Molo ($P \leq 0.05$). Clone Ks/71/6 had the widest bush diameter while clone Sb/66/107 had the narrowest bush diameter in both locations of 45.0 cm and 31.5 cm at Molo and 41.2 cm and 33.9 cm at Kabete. The parents had a wider bush diameter at Molo than at Kabete with mean value of 39.5 cm at Molo and 37.7cm at Kabete. However, clone Sb/66/107, P4 and Ks/70/64 had wider bushes at Kabete than at Molo (Table 8). Heterosis above high parent for bush diameter ranged between –11.2% to 41.3% and -31.3% to 27.6% for Kabete and Molo, respectively (Table 10).

Table 8: Mean first year performance of six pyrethrum clones at Kabete and Molo

	Bush diameter (cm)		Plant height (cm)		Dry flower weight (%)		Flower yield (kg/ha)		Pyrethrin content (%)		Pyrethrins yield (kg/ha)	
Clone	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
Sb/66/107	33 9	31.5	35.2	41.4	22.8	19.9	497.2	488.6	1 4	1.7	7 0	8 1
P4	37.0	36.9	45.5	54.6	22.2	21.0	624.5	593.0	1.5	1.8	9 4	10 5
Ks/74/122	35.0	43.9	41 8	45.0	22 8	20.5	487 4	503.9	1 4	1.6	7 0	8 3
Ks/71/6	41.2	45.0	44 4	50 8	21.7	20.3	613 6	540.2	1.5	1.6	9 0	8 7
L/64/4331	35 8	44.2	38 9	51.3	22.7	20 1	618 8	477.5	1.5	1.5	9 2	7 2
Ks/70/64	36 9	35 8	47.1	43 9	22 0	20.21	731.1	646 6	1 5	1.6	10 8	10 4
Mean	37.7	39 6	42.1	47.8	22 4	20.3	595 4	541 6	1.5	1.6	8 4	8 8
$LSD_{(0\ 05)}$	11 4	8 8	14.1	10 8	4 7	6 9	109 4	84 1	0 1	0 07	3 5	1 6

Table 9: Mean first year performance of 15 F_1 hybrids and their reciprocals at Kabete and Molo

Cross	Bush diameter (cm)		Plant height (cm)		Dry flower weight (%)		Flower Yields (kg/ha)		Pyrethrins cont. (%)		Pyrethrins yield (kg/ha)	
	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
Sb/66/107 x P4	44.5	39.8	59.6	41.4	20.6	20.7	899.1	846.3	1.5	1.4	13.6	11.8
Reciprocal	41.9	32.3	53.8	54.8	21.1	20.9	686.3	691.3	1.5	1.6	10.3	11.1
Sb/66/107 x Ks/74/122	47.4	42.7	56.1	55.1	22.6	20.8	864.7	903.1	1.7	1.5	14.7	13.5
Reciprocal	44.7	40.3	46.5	45.3	19.0	20.1	940.0	788.2	1.5	1.6	14.1	12.6
Sb/66/107 x Ks/71/6	38.0	36.1	49	43.0	20.1	19.9	775.7	806.3	1.6	1.9	12.9	15.3
Reciprocal	36.6	38.0	34.4	39.4	20.8	20.9	873.3	741.8	1.5	1.7	13.1	12.6
Sb/66/107 x L/64/4331	39.8	36.4	44.3	36.4	19.0	20.4	1433.9	597.4	1.8	2.0	25.8	11.9
Reciprocal	44.0	40.9	56.5	41.7	21.4	20.9	884.6	623.1	1.3	1.6	11.5	10.0
Sb/64/107 X Ks/70/64	38.8	35.3	49	43.6	20.8	20.5	686.7	618.8	1.5	1.5	10.3	9.3
Reciprocal	34.2	37.9	43.3	41.2	19.8	20.3	880.0	727.2	1.5	1.7	13.2	12.4
P4 x Ks/74/122	44.1	34.6	50.5	49.5	22.9	20.1	989.5	854.7	1.9	1.7	18.8	14.5
Reciprocal	40.9	35.6	47	49.0	21.7	20.3	960.2	809.9	1.5	1.8	14.4	14.6
P4 x Ks/71/6	40.6	38.1	52.3	42.5	21.1	20.0	656.3	797.5	1.6	1.7	10.5	13.6
Reciprocal	38.9	31.5	49	48.1	22.0	20.6	1040.3	733.9	1.5	1.5	15.6	11
P4 x L/64/4331	44.0	32.5	39.5	44.8	23.9	21.0	807.1	561.9	1.4	1.5	11.3	8.4
Reciprocal	45.5	36.0	41.9	45.5	20.8	20.6	585.7	818.8	1.4	1.6	8.2	13.1

Table 9 continues

P4 x Ks/70/64	50.0	47.1	61.8	51.3
Reciprocal	44.1	39.2	46	45.3
Ks/74/122 x Ks/71/6	39.2	33.7	47.5	42.1
Reciprocal	45.5	41.4	58.3	44.7
Ks/74/122 x L/64/4331	4.8.0	38.8	41.1	54.7
Reciprocal	50.5	48.6	56.2	49.5
Ks/74/122 x Ks/70/64	43.1	38.7	55.8	44.4
Reciprocal	43.9	30.1	57.9	53.4
Ks/71/6 x L/64/4331	41.3	39.9	52.8	51.2
Reciprocal	39.4	36.4	49.3	49.8
Ks/71/6 x Ks/70/64	42.2	38.9	46.9	46.7
Reciprocal	37.4	32.5	44	44.6
L/64/4331 x Ks/70/64	39.9	33.9	54.3	47.2
Reciprocal	39.3	35.8	30.2	44.6
Mean	40.8	37.4	49.2	46.4
$LSD_{(0.05)}$	10.8	10.5	15.2	13.4

17.7	20.7	1146.2	700.8	1.3	1.3	14.9	9.1
22.0	21.5	1178.6	584.4	1.4	1.4	16.5	8.2
20.7	20.0	957.1	733.0	1.4	1.6	13.4	11.7
21.6	20.8	918.8	868.8	1.6	1.7	14.7	14.8
22.0	20.2	875.0	1008.5	1.6	1.6	14.0	16.1
22.0	20.2	1231.8	1181.3	1.6	1.5	18.9	17.7
21.4	21.1	986.7	828.9	1.5	1.6	14.8	13.3
22.4	21.3	956.3	782.8	1.6	1.8	15.3	14.1
22.9	21.0	625.0	475.7	1.2	1.7	7.5	8.1
22.7	20.3	621.4	508.9	1.4	1.6	8.7	8.1
22	20.1	718.8	583.6	1.6	1.4	11.5	8.2
21.7	20.8	700.4	608.3	1.4	1.8	9.8	10.9
19.0	21.3	1056.3	712.3	1.6	1.5	16.9	10.7
20.9	20.2	1060.0	855.4	1.5	1.5	15.9	12.8
21.2	20.6	907.6	747.7	1.5	1.6	13.7	12.0
3.5	1.6	353.0	189.9	0.2	0.3	4.3	2.0

Table 10: Percent F_1 heterosis above high parent values in 30 pyrethrum crosses grown at Kabete and Molo

	Plant height (cm)		Bush diameter (cm)		Dry flower weight (%)		Flower yields (kg/ha)		Pyrethrins content (%)		Pyrethrins yield (kg/ha)	
Crosses	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
Sb/66/107 x P4	31.1	-24.1	20.3	8.0	-9.7	-1.3	44.0	42.7	2.0	-20.9	45.2	13.2
Reciprocal	18.4	.0.5	12.6	-12.4	-7.7	-0.2	9.9	16.6	0.0	-11.3	9.3	3.6
Sb/66/107 x Ks/74/122	34.2	22.5	35.3	-2.6	-1.1	1.7	88.6	79.2	14.6	-8.5	108.8	64.3
Reciprocal	11.2	0.8	27.7	-8.1	-16.8	-2.1	84.9	56.4	6.9	-3.0	99.9	41.4
Sb/66/107 x Ks/71/6	10.4	-15.4	-7.8	-19.8	-12.1	-1.9	26.4	107.0	6.1	13.9	41.5	143.7
Reciprocal	-22.4	-22.5	-11.2	-15.6	-8.8	3.4.	38.1	37.3	4.1	5.5	44.3	49.4
Sb/66/107 x L/64/4331	13.9	-29.1	11.3	-17.7	-16.8	1.6	132.9	22.3	18.8	8.5	179.3	43.6
Reciprocal	45.4	-18.6	22.9	-7.3	-6.3	4.1	46.0	27.5	-15.4	-1.8	24.4	23.4
Sb/66/107 x Ks/70/64	4.0	-0.8	5.0	-1.4	-8.7	1.6	-5.5	-4.3	0.7	-9.7	-4.7	-11.4
Reciprocal	-8.1	-6.1	-4.8	6.0	-13.3	0.7	23.6	12.5	-2.0	0.0	21.4	14.2
P4 x Ks/74/122	11.1	-9.4	19.1	-21.1	0.3	-4.3	58.2	44.1	27.3	-4.5	100.4	38.5
Reciprocal	3.4	-10.2	10.5	-18.9	-5.0	-3.1	57.6	36.6	-2.0	3.4	53.6	40.9
P4 x Ks/71/6	15.1	-22.2	-1.5	-15.3	-5.2	-4.4	8.6	34.5	4.0	-3.4	12.4	29.5
Reciprocal	7.8	-11.8	-5.5	-30.1	-1.0	-1.8	68.4	23.8	0.0	-14.1	66.7	6.5
P4 x L/64/4331	-13.1	-17.9	18.9	-26.5	7.7	0.2	33.5	-5.3	-10.0	-14.7	20.9	-19.3
Reciprocal	-7.9	-16.6	23.0	-18.5	-6.4	-1.8	-4.4	38.1	-10.0	-9.0	-12.4	25.7

Table 10 continues

P4 x Ks/70/64	31.2	-5.9	34.9	27.6
Reciprocal	-2.3	-16.9	19.2	6.3
Ks/74/122 x Ks/71/6	7.1	-17.1	-4.9	-25.2
Reciprocal	31.5	-12.0	10.5	-8.0
Ks/74/122 x L/64/4331	-1.6	6.7	14.1	-12.2
Reciprocal	34.5	-3.4	41.3	10.0
Ks/74/122 x Ks/70/64	18.4	-1.2	16.8	-11.7
Reciprocal	22.9	18.7	18.8	-31.3
Ks/71/6 x L/64/4331	19.0	.0.1	0.3	-11.4
Reciprocal	11.2	-2.9	-4.2	-30.3
Ks/71/6 x Ks/70/64	-0.4	-8.1	2.5	-13.5
Reciprocal	-6.6	-12.2	-9.3	-27.6
L/64/4331 x Ks/70/64	15.2	-8.0	8.0	-23.2
Reciprocal	-35.9	-13.0	6.3	-18.9
Mean	10.3	-5.37	11.02	-12.1

-20.5	-1.3	57.6	8.4	-13.3	-25.4	37.8	-11.6
-1.1	2.4	61.1	-9.6	-7.3	-19.2	52.0	-20.2
-9.3	-2.5	52.1	35.7	-3.4	-0.6	47.2	44.3
-5.2	1.6	54.8	60.8	4.7	3.1	62.1	69.7
-3.6	-1.6	44.8	100.1	4.7	-1.2	51.7	95.8
-3.4	-1.3	99.1	88.2	5.4	-11.0	108.4	65.2
-6.4	2.7	37.7	28.2	-1.3	-4.3	36.1	24.9
-1.8	3.9	38.5	21.1	4.0	10.4	40.9	36.6
3.4	3.4	-2.6	-12.0	-16.8	3.8	-18.5	-8.3
2.3	0.2	-2.1	-5.8	-3.4	0.0	-5.4	-5.2
-0.4	-1.0	-1.6	-9.7	8.1	-11.9	6.1	-20.1
-1.5	2.4	-7.1	-5.9	-3.4	10.0	-9.9	2.9
-14.3	5.5	49.5	10.2	4.0	-9.3	55.5	-0.1
-5.9	0.2	41.6	32.3	2.7	-5.0	46.2	28.8
-4.84	1.04	37.39	24.43	1.0	-4.33	36.44	21.62

Only hybrids Sb/66/107 x P4, P4 x Ks/70/64 and Ks/74/122 x L/64/4331 and their reciprocals had positive heterosis for bush diameter in both locations. Heterosis for bush diameter was better expressed at Kabete than at Molo. Twenty-two crosses at Kabete and five crosses at Molo out of the 30 crosses expressed positive heterosis. Hybrid L/64/4331 x Ks/74/122 expressed the highest heterosis of 41.3% at Kabete while hybrid Ks/70/64 x P4 expressed the highest heterosis of 27.6% at Molo. Heterosis was not consistent in the two locations. Most hybrids expressed negative heterosis at Molo but positive heterosis at Kabete with mean percentage heterosis of 10.7% at Kabete and –12.0% at Molo (Table 10).

4.7.2 Plant height

There were no significant differences in plant height among the parental clone in both locations ($P \geq 0.05$). Clone P4 was the tallest at Molo while clone Ks/70/64 was the tallest at Kabete with plant heights of 54.6cm and 47.1cm, respectively (Table 8). Parent Sb/66/107 was the shortest in both locations with a plant height of 35.2cm at Kabete and 41.4 cm at Molo. All the parents were taller at Molo than at Kabete except for clone Ks/70/64 (Table 8). Hybrids Sb/66/107 x Ks/74/122 and its reciprocal, P4 x Sb/66/107 and Ks/70/64 x Ks/74/122 expressed positive heterosis above high parent values in both locations (Table10). The tallest and shortest hybrids were P4 x Ks/70/64 and Ks/70/64 x L/64/4331, respectively when grown at Kabete. At Molo the tallest and shortest hybrids were Sb/66/107 x P4 and Ks/71/6 x Sb/66/107, respectively. The hybrids were taller at Kabete than at Molo. Out of the 30 crosses, 21 crosses at Kabete and five crosses at Molo showed positive heterosis. Heterosis for plant height varied from –35.9% to 45.4%. The highest heterosis of 45.4% was expressed by hybrid L/64/4331 x Sb/66/107 when grown at Kabete. The lowest heterosis for plant height of –35.9% was expressed by Ks/70/64 x

L/64/4331 when grown at Kabete. Heterosis was not consistent in both locations. Mean heterosis was 9.6% at Kabete and –8.3% at Molo (Table10).

4.7.3 Percent dry flower weight

There were no significant differences in flower dry weight among the parental clones in both locations ($P \geq 0.05$). Percent dry flower weight ranged from 19.9% to 22.8% (Table 8). Parent Ks/74/122 expressed the highest dry flower weight at Kabete while P4 expressed the highest at Molo of 22.8% and 21.0%, respectively. Clones Ks/71/6 at Kabete and Sb/66/107 at Molo expressed the lowest dry flower weight of 21.7% and 19.9%, respectively. All the parents had a higher dry flower weight at Kabete than at Molo with averages of 22.4% and 20.3%, respectively (Table 9).

Heterosis for dry flower weights ranged from –16.8% to 7.7% (Table 10). Only crosses P4 x L/64/4331, Ks/71/6 x L/64/4331 and its reciprocal showed positive heterosis for dry flower weight in both locations of 7.7% and 0.2%, 3.4% and 3.4%, and 2.3% and 0.2% at Kabete and Molo respectively. Four crosses at Kabete and 16 crosses at Molo out of 30 crosses showed positive heterosis. This means that most of the crosses that had positive heterosis at Molo had negative heterosis at Kabete. This indicates that environment affected expression of heterosis. Heterosis ranged between –16.8% to 7.7%. The highest heterosis (7.7%) was expressed by P4 x L/64/4331 while the lowest heterosis of –16.8% was expressed by Sb/66/107 x L/64/4331 when grown at Kabete (Table 10).

4.7.4 Dry flower yields

There were no significant differences in dry flower yields among the parents at Kabete ($P \geq 0.05$). At Molo there were significant differences among the parents ($P \leq 0.05$)

Parental dry flowers yields ranged from 477.5 kg/ha expressed by clone L/64/4331 grown at Molo to 731.1 kg/ha expressed by clone Ks/70/64 grown at Kabete (Table 8). Ks/70/64 was the best yielder at both locations, yielding 731.1 kg/ha at Kabete and 646.6 kg/ha at Molo. Clone Ks/74/122 had the poorest yields at Kabete while L/64/4331 at Molo with yields of 487.4 kg/ha and 477.5 kg/ha, respectively. All the parental clones gave better yields at Kabete than at Molo except for parent Ks/74/122 (Table 8). Hybrid L/64/4331 x Ks/74/122 at Kabete while Sb/66/107 x Ks/71/6 at Molo had the highest dry flower yields of 1,231.8 kg/ha and 1,118.3 kg/ha, respectively (Table 9). Hybrid L/64/4331 x Ks/71/6 at Molo had the lowest dry flower yield of 509.kg/ha. The hybrids gave higher yields at Kabete than at Molo. Heterosis for dry flower yield ranged from –12.0% to 132.9% (Table 10). Cross Sb/66/107 x L/64/4331 grow at Kabete while cross Sb/66/107 x Ks/71/6 at Molo expressed the highest percent heterosis of 132.9% to107.0 %, respectively. Twenty-two out of 30 hybrids had positive heterosis above high parent values for flower yields. Hybrids Sb/66/107 x Ks/71/6, Sb/66/107 x L/64/4331 and Ks/74/122 x L/64/4331 had heterosis over 100% in at least one location (Table 10).

4.7.5 Pyrethrins content

There were no significant differences in pyrethrins content among the parents at both locations ($P \geq 0.05$). Pyrethrins content ranged between 1.4% to 1.8% (Table 8). Clone P4 was the best performer in both locations with pyrethrins content of 1.8% at Molo and 1.5% at Kabete. Clone Sb/66/107 at Kabete and L/64/4331 at Molo had the lowest percent pyrethrins content of 1.4% and 1.5%, respectively. All the parents performed better at Molo than at Kabete (Table 8). Pyrethrins content in hybrids ranged between 1.24% to 2.0% (Table 9). Hybrids had higher pyrethrins content at Molo than at Kabete. Hybrids Sb/66/107 x L/64/4331 at Molo and P4 x Ks/74/122 at Kabete had the highest percent

pyrethrins content of 2.0% and 1.9%, respectively (Table 9). The hybrids had higher pyrethrins content at Molo than at Kabete. Heterosis in pyrethrins content ranged between –25.4% to 27.3% (Table 10). Only hybrids Sb/66/107 x Ks/71/6 and its reciprocal, Sb/66/107 x L/64/4331, Ks/71/6 x Ks/74/122, and Ks/70/64 x Ks/74/122 showed positive heterosis in both locations. At Kabete, 16 crosses while eight crosses at Molo out of 30 crosses had positive heterosis (Table 10).

4.7.6 Pyrethrins yield

There were no significant differences in pyrethrins yield (P≥ 0.05) among the parental clones at Kabete. At Molo, there were significant differences in yields among the parents at (P≥ 0.05). Pyrethrins yield ranged between 7.0 kg/ha to 10.8 kg/ha among the parental clones (Table 8). Clone Ks/70/64 had the highest pyrethrins yield at Kabete while Clone P4 was the best at Molo with pyrethrins yield of 10.8 kg/ha and 10.5 kg/ha, respectively. Clones Ks/74/122 at Kabete and L/64/4331 at Molo had the lowest pyretrins yield of 7.0 kg/ha and 7.2 kg/ha, respectively (Table 8). The parents performed better at Molo than at Kabete except for clones Ks/71/6, L/64/4331 and Ks/70/64 (Table 8). Heterosis ranged between –20.4% to 179.3% (Table 10). Hybrid Sb/66/107 x L/64/4331 expressed the highest heterosis of 179.33% at Kabete while Sb/66/107 x Ks/71/6 expressed the highest heterosis of 143.7% at Molo. Among the hybrids, pyrethrins yield ranged between 7.5 kg/ha to 25.9 kg/ha. The highest yielder was Sb/66/107 x L/64/4331 and the lowest yielder was Sb/66/107 x Ks/74/122 grown at Kabete (Table 9). Twenty out of 30 crosses had positive heterosis in both locations. Hybrids Sb/66/107 x Ks/74/122, Sb/66/107 x L/64/4331, P4 x Ks/74/122, and L/64/4331 x Ks/74/122 had heterosis of over 100% (Table 10). It can be noted that three out of the four crosses with heterosis over 100%

have clone Ks/74/122 as one of their parents. Most of the crosses that expressed negative heterosis for this trait involved clone Ks/70/64 as one of their parents.

4.8 General combining ability

General combining ability (GCA), specific combining ability (SCA), and reciprocal mean of squares. GCA effects, SCA effects and reciprocal effects were worked out following the procedure shown in Appendix A (a worked example on pyrethrins yields at Kabete). The results of a 6 x 6 diallel are presented in Tables 11, 12, 13, and 14.

4.8.1 Bush diameter

The GCA mean squares for bush diameter were not significant in both locations. SCA mean squares were significant at both locations. Reciprocal mean squares were only significant at ($P \leq 0.05$) at Molo but not at Kabete. The GCA to SCA ratios were 0.56 and 0.76 at Kabete and Molo respectively. This means that there was environmental effect on bush diameter (Table 11). Clones P4 and Ks/74/122 at Kabete and only Ks/74/122 at Molo expressed significant positive GCA effects. Clone Ks/74/122 was the best general combiner at both locations with GCA effects of 1.36 and 1.57 at Kabete and Molo respectively. Clones Sb/66/107 and P4 were the poorest general combiners with GCA effects of –1.26 and –0.88 at Kabete and Molo respectively (Table 12).

Table 13 shows that only hybrids Sb/66/107 x Ks/74/122 and P4 x Ks/70/64 had significant specific combining ability at Kabete while P4 x Ks/70/64 was the only significant specific combination at Molo ($P \leq 0.05$). Hybrid P4 x Ks/70/64 was the best specific combination at both locations with SCA effect of 5.19 and 7.45 at Kabete and Molo respectively.

Table 11: GCA, SCA and reciprocal mean squares of six pyrethrum clones and their F_1s at Kabete and Molo

Source	Df	Plant height		Bush diameter		Flower yield		Pyrethrins content		Pyrethrins yield	
		Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
GCA	5	127.7**	36.8*	15.2	16.5	38672.6**	22750 8**	0.02**	0.02**	9 2**	8 0**
SCA	15	30.8	23.2**	23.5**	10.4**	78059.3**	40534.7**	0.01*	0.02**	21 5**	13.2**
Reciprocal	15	58.3**	17.3	8.6	16.6*	23381.0**	10778 6**	0.02**	0 02**	10 5**	4 6**
Error	70	30 9	24 0	24.8	14.7	8920.2	2567 4	0.01	0.01	2 3	0 5
GCA:SCA		0 89	0.76	0 56	0 76	0 50	0 53	0.80	0 67	0 46	0 55

*,** = Significant at 0.05 and 0.01 probability levels respectively

Table 12: Estimates of General combining ability (GCA) effects of six pyrethrum clones at Kabete and Molo.

	Bush diameter		Plant height		Flower yield		Pyrethrins content		Pyrethrins yield	
Parent	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
Sb/66/107	-1.3	-0.9	-1.0	-2.9	-22.4	6.0	0.01	0.03	-0.09	0.23
P4	1.2	-1.1	1.4	1.8	2.3	2.1	-0.01	-0 02	-0.13	-0 10
Ks/74/122	1.4	1.6*	1.7	1.5	47.3**	81.1**	0.05*	0.03	1.04**	1 44**
Ks/71/6	-1.0	0.2	-0.2	-0.5	-100 0	-25 9	-0.01	0.04	-1 55	0 03
L/64/4331	0 2	1.2	-2.6	0.7	17 4	-41.3	0 03	-0 03	0 23	-0 89
Ks/70/64	-0 5	-1.0	0.7	-0.8	55.4**	-22.1	-0 02	-0 05	0 50*	-0 70

*, ** Significant at 0.05 and 0.01 probability levels respectively

Table 13: Estimates of Specific combining ability (SCA) effects of 15 F_1 hybrids of pyrethrum based on their mean performance at Kabete and Molo.

Cross	Bush diameter		Plant height		Flower yield		Pyrethrins content		Pyrethrins yield	
	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
Sb/66/107 x P4	2.1	0.3	8.3*	2.6	-42.8	47.3	0 01	-1.40	-0 7	-0.3
Sb/66/107 x Ks/74/122	4.8*	3.1	2.6	5.0	48.0	45.1	0 03	-0.12	0.5	-0.5
Sb/66/107 x Ks/71/6	-1.6	-0.1	-5.0	-2.1	78.4	36 5	0 05	0 13*	1.8	5 2**
Sb/66/107 x L/64/4331	1.8	0.6	6.0	-5.4	321.7**	-67.9	0 03	0 18*	5 6**	-0.1
Sb/66/107 x Ks/70/64	-2.4	0.7	-1.5	-0.6	-91.6	-24.3	-0 02	-0 02	-1 5	-0.5
P4 x Ks/74/122	-1.2	-3.2	-2.4	-0.8	81.1	35 7	-1.35	0 14*	2 8*	1.8
P4 x Ks/71/6	-1.6	-2 2	1.5	-2.7	105.5	76 1	-1 45	-0.02	0 9	0 9
P4 x L/64/4331	2.2	-3.6	-6.1	-4.0	-159 8	26 2	-0 12	0 01	-3 2	0 3
P4 x Ks/70/64	5 2*	7 5*	33.8**	0.6	251.7**	-50 8	-0.13	1 21**	2 5*	-1 9
Ks/74/122 x Ks/71/6	0.9	-2.1	3.4	-4.3	138 7*	32 3	-0 05	-0 02	1.7	0 6
Ks/74/122 x L/64/4331	3 0	3 2	1.6	3 2	143.4*	225 1**	0 05	0 08	2 3*	2 9*
Ks/74/122 x Ks/70/64	1.5	-3 9	6 4	0.5	51.5	34 4	-0 02	0.10	0 6	1.4
Ks/71/6 x L/64/4331	0.1	-1.1	5 9	3 6	-168 9	-154	0 12*	0 01	-3 4	-2 6
Ks/71/6 x Ks/70/64	0.2	-1.3	-3 0	0.3	-111.7	-69 5	0 05	-0 01	-1.2	-1 3
L/64/4331 x Ks/70/64	-1.0	-3 1	-3 9	-0.7	135 3*	133 8*	0 09	-0 04	2 7*	2 0

*, ** Significant at 0.05 and 0.01 probability levels respectively.

Table 14: Estimation of reciprocal effects of 15 F_1 hybrids of pyrethrum based on their mean performance at Kabete and Molo.

Cross	Bush diameter		Plant height		Flower yield		Pyrethrins content		Pyrethrins yield	
	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo	Kabete	Molo
Sb/66/107 x P4	1.3	3.8	2.9	-6.7	106 4	77.5	0.02	-0.09	1 7*	0 5
Sb/66/107 x Ks/71/6	0.7	-1.0	7.3*	1.8	-35.8	188.3**	0 02	0.07	-0.1	4 1**
Sb/66/107 x L/64/4331	-2.1	-2.3	-6.1	-2.7	269.0**	-12.8	0.26**	0.18*	7.2**	0 8
Sb/66/107 x Ks/70/64	1.8	-1.3	2.8	1.2	-106.3	-54.2	0.02	-0.08	-1 4	-1.3
P4 x Ks/74/122	1.6	-0.5	1.8	0.2	1.9	22.4	0.22**	-0.07	2.2**	-0.1
P4 x Ks/71/6	1.2	3.3	1.7	-2.8	-188.0	31.8	0.03	0 10	-2.6	1 2*
P4 x L/64/4331	-0 8	-1.8	-1.2	-0.4	118.2	-128 5	0 00	-0 05	1 6*	-2 4
P4 x Ks/70/64	2 9	3.9	7.9	3 0	-13.0	58.2	-0.05	-0 06	-0 8	0 5
Ks/74/122 x Ks/71/6	-3.2	-3.9	-5.5	-1.3	-8.1	-67.9	0.06	-0 03	-0 7	-1 1
Ks/74/122 x L/64/4331	-4 9	-4.9	-7.5	2 6	-168.1	30.2	-0 01	0 08	-2 5	1 3*
Ks/74/122 x Ks/70/64	-0 4	4.3*	-1.1	-4 5	-2.7	23 1	-0 04	-0 12	-0 3	-0 6
Ks/71/6 x L/64/4331	0 9	1.8	1.7	0 7	-1.5	-16.7	-0.10	0 03	-0 6	-0 1
Ks/71/6 x Ks/70/64	2 4	3.2	1.5	1.1	19 8	-12.3	0 09	-0 18	0 9*	-1 2
L/64/4331 x Ks/70/64	0.3	-0 9	12.1**	1.3	28 8	-71 6	0 01	-0 04	0 5	-1 5

*, ** Significant at 0.05 and 0.01 probability levels respectively

Crosses Sb/66/107x Ks/70/64 at Kabete and L/64/4331x Ks/70/64 at Molo were the poorest specific combiners with SCA effects of –2.35 and –3.07 respectively. Only hybrid Ks/74/122x Ks/70/64 expressed significant reciprocal effect at Molo with a value of 4.3. The other hybrid combinations had no significant reciprocal effects (both positive and negative) in both locations (Table 14).

4.8.2 Plant height

GCA mean squares for plant height were significant at both locations ($P \leq 0.05$). SCA mean squares were only significant at Molo. Reciprocal mean squares were only significant at Kabete. The GCA to SCA ratios were 0.86 and 0.76 at Kabete and Molo respectively. However, the clones expressed positive significant GCA effects for plant height in either location (Table11).

Table 12 shows that clone P4 was the best general combiner at Molo while clone Ks/74/122 was the best general combiner at Kabete with GCA effects of 1.83 and 1.71. respectively. Clone Sb/66/107 at Molo and L/64/4331 at Kabete were the poorest general combiners with GCA effects of –2.88 and –2.60 respectively. Only crosses Sb/66/107 x P4 and P4 x Ks/70/64 at Kabete expressed significant combining ability. Cross Sb/66/107x P4 was the best specific combination at Kabete while cross Sb/66/107 x Ks/74/122 was the best specific combination at Molo with SCA effects of 8.29 and 4.95 respectively. Crosses P4x L/64/4331 at Kabete and Sb/66/107 x L/64/4331 at Molo were the poorest specific combinations with SCA effects of –6.14 and –5.41 respectively (Table 13). It's worth noting that clone Sb/66/107 appears in the best specific combinations while clone L/64/4331 appears in the poorest specific combinations at both

locations. Only cross L/64/4331 x Ks/70/64 at Kabete expressed significant reciprocal effect (P≤ 0.05) (Table 14).

4.8.3 Flower yield

Table 11 shows that GCA means squares. SCA mean squares and reciprocal mean squares for fresh flower yield were highly significant at both locations (P≤ 0.05). The GCA to SCA ratios were 0.56 and 0.53 at Kabete and Molo respectively. Ks/70/64 at Kabete and Ks/74/122 at Molo were the best general combiners for fresh flower yield with largest significant GCA effects of 55.39 and 18.12 respectively. Clone Ks/71/6 at Kabete and L/64/4331 at Molo were the poorest general combiners with GCA effects of –100.00 and –41.28, respectively (Table 12).

Out of the 15 crosses, five at Kabete and two at Molo had significant positive SCA effects of which crosses L/64/4331 x Ks/70/64 and Ks/74/122 x L/64/4331 had significant SCA effects in both locations (P≤ 0.05). Cross P4 x Ks/70/64 at Kabete and Ks/74/122 x L/64/4331 at Molo were the best specific combinations. Cross Ks/71/6 x L/64/4331 was the poorest specific combination in both locations with negative SCA effects (Table13). Table 14 shows that only crosses Sb/66/107 x Ks/71/6 at Molo and Sb/66/107 x L/64/4331 when Sb/66/107 is used as male expressed significant positive reciprocal effects. Crosses P4 x L/64/4331 at Molo and P4 x Ks/71/6 at Kabete had the largest negative reciprocal effects.

4.8.4 Pyrethrins content

GCA means squares and reciprocal mean squares were highly significant for pyrethrins content in both locations. SCA mean squares were also significant at both locations (P≤

0.05). The GCA to SCA ratios was 0.80 and 0.67 at Kabete and Molo respectively (Tables11).

Table12 shows that only clone Ks/74/122 grown at Kabete expressed significant GCA effect ($P \leq 0.05$). Clones Ks/74/122 at Kabete and clone Ks/71/6 when grown at Molo were the best general combiners with GCA effects of 0.051 and 0.038 respectively. Clone L/64/4331 at Kabete and Ks/70/64 at Molo were the poorest general combiners for pyrethrins content. Crosses Sb/66/107 x Ks/71/6, Sb/66/107 x L/64/4331, P4 x Ks/74/122 and P4 x Ks/70/64 at Molo and only Ks/71/6 x L/64/4331 at Kabete had significant positive SCA effects. Crosses Ks/71 6 x L/64/4331 at Kabete and cross P4 x Ks/70/64 grown at Molo were the best specific combinations with SCA effects of 0.118 and 1.21 respectively. Crosses P4 x Ks/71/6 at Kabete and Sb/66/107 x P4 at Molo were the poorest specific combinations (Table 13.

Only crosses Sb/66/107 x L/64/4331 and P4 x Ks/74/122 at Kabete and Sb/66/107 x L/64/4331 at Molo had significant positive reciprocal effects when Sb/66/107 was used as male. It is worthy noting that Cross Sb/66/107 x L/64/4331 had significant reciprocal effect in both locations. Most crosses had positive reciprocal effects in one location and negative in the other. This could have been due to environmental effects. Crosses Ks/71/6 x L/64/4331 grown at Kabete and crosses Ks/71/6 x Ks/70/64 grown at Molo had the largest negative reciprocal effects (Table 14).

4.8.5 Pyrethrins yield

GCA mean squares, SCA mean squares and reciprocal mean squares were highly significant for pyrethrins yield in both locations ($P \leq 0.05$). The GCA to SCA ratios were

0.46 and 0.55 at Kabete and Molo respectively (Table 11). Clone Ks/74/122 was the best general combiner at both locations with significant positive GCA effects of 1.043 and 1.440 at Kabete and Molo. respectively. CrossL/64/4331 grown at Molo and clone Ks/71/6 grown at Kabete were the poorest general combiners with GCA effects of -0.894 and –1.548 respectively (Table12).

Cross Sb/66/107 x L/63/4331 at Kabete and cross Sb/66/107 x Ks/71/6 at Molo were the best specific combinations with SCA effects of 5.6 and 5.2 respectively. Cross Ks/71/6 x L/64/4331 was the poorest specific combination in both locations with the largest negative SCA effects of –2.6 at Molo and –3.4 at Kabete. Cross Ks/74/122 x L/64/4331 was the only one with significant SCA effects in the two locations (Table 13). Five crosses at Kabete and three at Molo had significant reciprocal effects. Cross Sb/66/107 x L/64/4331 at Kabete while Sb/66/107 x Ks/71/6 at Molo had the highest reciprocal effects of 14.3 and 8.2. respectively. Cross L/64/4331 x Ks/70/64 at Molo and Ks/74/122 x L/64/4331 at Kabete had the largest negative reciprocal effects of –3.1 and –4.9. respectively (Table 14).

CHAPTER FIVE

DISCUSSION

5.1 Compatibility test

All the six clones in this study produced viable pollen grains with percent germination ranging from 70.4% to 80.4% after 96 hours. Production of viable pollen is important when choosing parent and testing them for hybridization. Stanley and Linskens (1974) observed that pollen grains with viability of over 40% gave good seed set. In this study, given that pollen viability was over 70%, failure of the clones to set viable seeds after crossing or selfing implies that the clones are cross- or self-incompatible.

The six clones studied produced viable hybrid seeds with percent pure germinating seeds ranging from 5.7% to 45.7%. Brewer (1968) using pair-wise planting of parents in isolated plots method obtained percent pure germinating seeds ranging from 5.2% to 27.5%. Parlevliet and Brewer (1971) and Garstel (1950) observed that sporophytic self-incompatibility system operates in pyrethrum. Clones with identical genetic constitution could not cross to produce viable hybrid seeds. The production of viable hybrid seeds in this study implies that the six clones studied were cross compatible and were genetically dissimilar at least at the self-incompatibility (S- locus) and crossability loci

No seeds produced after self-pollination were viable. However Brewer (1968), successfully obtained selfed seeds from Congo 2388, 2449, 5227 and 5272 pyrethrum clones through forced self-pollination using cellophane bags. Parlevliet and Brewer (1971) and Garstel (1950) observed that in pyrethrum sporophytic self-incompatibility system operates. The failure to produce viable seeds in this study after self-pollination indicates that the six clones were self-incompatible consistent with the earlier reports that

sporophytic self- incompatibility system operates in pyrethrum including the six clones used in this study.

Manual crossing method where only the ray florets are used has never been reported in pyrethrum. However, this method has been employed in other crops with inflorescent flowers. Failure to have effective pollination leads to poor or no seed set (Watts, 1980). Brewer (1968) observed that in pyrethrum conditions that hinder insect movement like cold environmental conditions adversely affected the quality of seeds produced. Production of viable seeds after employing manual crossing of ray florets only implies that the method is effective and can be used in a hybridization program.

Despite the success obtained in this study using manual crossing of ray florets only, the method has disadvantages compared to pair-wise planting of parents in isolated plots. In this study, one inflorescence had 14 to 22 ray florets and a total of 118 to 188 florets (ray plus disc florets). In cases where pair-wise planting of parents in isolated plot method is used, seeds from the whole inflorescent are harvested (Ndambuki, 1979; Brewer, 1968). When manual crossing of ray florets method is used, only seeds from the ray florets are harvested. In this study, assuming 100% seed set, manual crossing of ray florets only would yield between 14 to 22 seeds compared to 118 to 188 seeds in case of pair-wise planting of parents in isolated plots is used. Therefore, manual crossing of ray florets only is laborious and time consuming.

Pollen grains germination and pollen tubes growth were observed in all the crosses and none in the selfs in this study using fluorescent microscopy method. Drelow *et al* (1974) working on *Chrysanthemum morifolium* observed that in most members of Compositae

family which exhibit self-incompatibility, inhibition of pollen tubes occurred at the stigmatic surface. Only compatible pollen grains remain attached to the stigma after cross-pollination. Heslop-Harrison (1971) observed that in sporophytic incompatibility, pollen grains germination on the stigma was inhibited or there was interference with the early stages of tube growth while still on the stigma. Brewer (1968) observed that in pyrethrum, self-fertilization was prevented by inhibition of pollen grains germination on the stigma. The observation of pollen grain germination on stigmas and pollen tube growth in the styles in this study confirms that the clones were cross compatible. Failure to observe pollen grains germination on the stigmas and subsequent pollen tubes in the styles after self-pollination implies that the clones were self-incompatible confirming that sporophytic self-incompatibility system operates in the clones studied.

The existence of sporophytic self-incompatibility system in pyrethrum as suggested from the results of this study indicates that no selfed seeds can be produced under normal conditions in the field. Hybrids seeds can be produced by pairwise planting of the parents in isolated plots as described by Ndambuki (1979).

5.2 Heterosis

All the six-pyrethrum parental clones performed better at Molo (2450m) than at Kabete (1820m) except for flower yield. Pyrethrins content for the parents and the hybrids were higher at Molo than at Kabete. Ikahu and Ngugi (1988) observed that flower yield and pyrethrins content were highly affected by temperature. They demonstrated that flower yields and pyrethrins contents increased with increase in altitude. The results of this study concur with these observations except for flower yields among the parents.

The magnitude and sign of heterosis observed under this study varied much from one location to another for all the characters studied. These variations are indication of environmental effects. The hybrids and their parents were affected differently under different environmental conditions. For example hybrid P4 x L/64/4331 had heterosis value of –19.3% at Molo while at Kabete it had 20.9% for pyrethrins yield. L/64/4331 x P4 had 38.1% at Molo and –4.4% at Kabete for flower yield. These results concur with observation by Ikahu and Ngugi (1988). They observed that environmental factors such as rainfall, temperature and soil affected different clones differently.

About 80% of the hybrids expressed positive heterosis for flower and pyrethrins yields. Heterosis for pyrethrins content was low in magnitude irrespective of the sign. Singh and Sharma (1989) observed positive heterosis for flower yields but low values and mostly negatives for pyrethrins yields and contents after crossing 12 pyrethrum clones. Positive heterosis observed in this study in flower and pyrethrins yields indicates that hybrids with good gene combinations can be produced after crossing pyrethrum. This can lead to enhanced yield performance in pyrethrum clones in Kenya. Hybrids such as Sb/66/107 x Ks/71/6 and Sb/66/107 x L/64/4331 for flower and pyrethrins yields at Molo and Kabete respectively. Ks/74/122 x L/64/4331 for flower yields at Molo, Sb/66/107 x Ks/74/122. P4 x Ks/74/122 and L/64/4331 x Ks/74/122 for pyrethrins yield at Kabete had heterosis of over 100%.

The hybrids expressed heterosis values of low magnitude for pyrethrins content, most of them being negative. These results concur with those observed by Singh and Sharma (1989). They observed that 90% of the hybrids after crossing 12 pyrethrum clones

expressed negative heterosis for pyrethrins content. High heterosis (regardless of sign) in hybrids indicates high genetic diversity between the two parents involved (Mungoma and Pollak, 1988 and Singh and Sharma, 1989). Therefore, it implies that the clones used in this study had low genetic diversity for pyrethrins content improvement of pyrethrins yields which is a function of flower yields and pyrethrins content can be attained by exploiting the genetic diversity observed on flower yields.

The best performing hybrids did not necessarily come from crosses between the best parents. For example, cross Sb/66/107 x Ks/74/122 at Kabete had a heterosis percentage of 108.8% for pyrethrins yield yet the two parents had the lowest pyrethrins yield. Cross Sb/66/107 x L/64/4331 at Kabete had heterosis of 179.3% for pyrethrins yield yet Sb/66/107 and L/64/4331 were not the best yielders. Contant [1963(b)] realized that selection of high yielding clones and their subsequent use in hybrid clones did not necessarily give superior progenies. He however noted that testing for breeding value (BV) and then using parents with high BV gave better progenies in a fairly short time. This observation is confirmed in this study. Thus clone Ks/74/122, which had the highest significant GCA effects for bush diameter, flower yields, pyrethrin contents and pyrethrins yield produced hybrids with high positive heterosis.

5.3 Combining ability

General combining ability (GCA) and specific combining ability (SCA) mean squares were significant for all the traits except bush diameter in both locations. The average performance of a single-cross progeny in a diallel cross is broken into components relating to general combining ability (main or additive gene effects) and to specific

combining ability (interactions or non-additive gene affects). If the specific combining ability mean squares are not significant, then the performance of a single-cross progeny can be adequately predicted based on general combining ability. Crossing the two parents having the highest general combining ability may produce the best performing progeny. If SCA mean squares are significant, then there is need for determining the importance of the interactions in determining the performance of the progeny (Baker, 1978). Ndambuki (1979) observed that flower yields, pyrethrins content and pyrethrins yields were all under additive gene effects. He found similar results after using diallel cross, polycross and topcross methods. However, he estimated general combining ability values as array means while specific combining ability values as actual F_1 values.

The significance of GCA and SCA mean squares under the present study suggests that both additive and non-additive gene effects were responsible for the manifestation of variability for these traits. In a situation where both gene effects are important, exploitation and fixation of additive gene effects can be attained by selecting superior parental clones for hybridization, followed by more intensive selection of superior hybrids in advanced generations (Tumwesigye, 1988).

In cases where SCA mean squares are significant, total genetic variance among single cross progeny is equal to twice the GCA component plus the SCA component of variance (Baker, 1978). Assessment of progeny performance is then determined by estimating the component of variance and expressing them in the ratio of two times the variance due to GCA over total variance (variance due to GCA plus SCA). The closer this ratio is to unity the greater the predictability based on GCA alone (Baker, 1978). In this study, All the traits studied showed a ratio of above 0.50 except for pyrethrins yields at Kabete. Plant

height expressed the highest ratios followed by pyrethrins contents. These high ratios are indicative of the significant effects of GCA (additive effects) on the performance of the traits. Therefore, prediction of the progeny performance these traits with high GCA to SCA ratio can be based on GCA of the parents (Tumwesigye, 1988; Baker.1978)

Significance of SCA mean squares indicates that the traits were under non-additive gene effects. Significance of SCA mean squares is as a result of interaction between genes from the two parents within the loci. This interaction leads to heterosis whose magnitude depends on how diverse the two parents are at a given locus or trait (Brandle and McVetty, 1985; Falconer, 1979). Non-additive gene effects can be exploited by superior single hybrid plant selection (Tumwesigye, 1988). Pyrethrum is propagated either sexually by seeds or vegetatively by splits. Superior specific pyrethrum hybrid combinations as a result of non-additive gene effect can be maintained and multiplied by vegetative propagation. Through this method, the genetic constitution of the hybrids is maintained at the same time non-additive genes are exploited.

All the traits except plant height and bush diameter expressed significant reciprocal effects. Significant reciprocal effects suggest that the direction of the cross matters. It further suggests that there are cytoplasmic factors that influence the expression of these characters. These factors because they are outside the nuclear chromosomes, they are referred to as extra-nuclear factors or cytogenes. This could be due to the DNA within other organelles outside the nucleus (cytoplasmic DNA) (Tumwesigye, 1988). These results differ from those obtained by Ndambuki (1979) where only GCA means squares were significant. However, similar observation have been made in other crops like in four-o'clock plant (*Mirabilis jalapa*). Extra-nuclear genes in this plant were observed to

influence the colour of their leaves between green, white and green-white (variegation). This extra-nuclear DNA was found to be in the cytoplasts (Strickberger, 1990; Simmonds, 1979 and Poehlman, 1987).

Clone Ks/74/122 was the best general combiner with significant positive GCA effects for all the traits except for plant height while clone Ks/70/64 had significant GCA effects for flower yield and pyrethrins yield. Contant [1963(b)] observed that determination of combining ability with subsequent crossing of the clones with high combining ability resulted in a faster achievement of genetic improvement. In this study clone, Ks/74/122 and Ks/70/64 used, as parents would lead to a faster improvement of yields.

Hybrids Ks/74/122 x L/64/4331 for flower and pyrethrins yields, and P4 x Ks/70/64 for pyrethrins content were the best combinations with significant SCA effects. It can be noted that the two hybrids had at least one their parent observed above as being the best general combiner. This observation concurs with that of Contant [1963(b)] in pyrethrum that parents with high GCA end up giving good hybrids for the trait.

Although some clones had low insignificant GCA effects, they gave very good combinations for certain traits. For example Sb/66/107 x L/64/4331 for flower and pyrethrins yields at Kabete. The two parents had insignificant GCA effects but produced a very good hybrid combination with significant SCA effects. Highly significant SCA effects and high heterosis in hybrids from parents with insignificant GCA effects is as a result of the action of non-additive gene effects and using genetically divergent parents (Brandle and McVetty, 1985; Tumwesigye, 1988). The two parents above are genetically divergent for the two traits. In addition, the two traits are under non-additive gene action.

CHAPTER SIX

CONCLUSIONS

1) Data presented in this study indicate that the six parental clones studied were cross compatible and self-incompatible. Observation of pollen grains germination on the stigma and pollen tubes growth in the styles using fluorescent microscopy technique confirmed that the six clones were cross compatible. In addition. failure of pollen grains to germinate on the stigmas after selfing suggests that sporophytic self-incompatibility operate in the six clones.

2) Manual crossing of the ray florets only was an effective method of crossing. However. the method was laborious and time consuming and therefore can not be employed in commercial seed production programs. The method is however very accurate and allows the breeder to be sure of the hybrids' parentage. It should be used in programs where only a few parents are to be crossed or tested for cross compatibility.

3) Data from the current study shows that heterosis above high parent value was observed in 22 and 21 out of 30 hybrids for flower yields and pyrethrins yields respectively, six out of 30 hybrids for plant height and four out of 30 hybrids for pyrethrins content and bush diameter regardless of location. Observation of heterosis especially for flower and pyretthrins yield implies that there is room for improvement on pyrethrum yields and production in Kenya at large.

4) Both additive and non-additive gene effects were observed to be responsible for manifestation of variability in all the traits studied except for bush diameter. The two gene effects can be exploited in pyrethrum because it is propagated by both sexual (using seedlings) and asexually (vegetatively by splits). Additive gene effects can be exploited by using sexual propagation by selecting superior parents for hybridization followed by more intensive selection of superior hybrids in advanced generation

(superior single plant selection). In doing this, along the line, hybrids that show exceptional performance can be taken for adaptability tests and released for commercial production while others can be incorporated back in the program as parents. Good performing hybrids which on crossing produce inferior progenies (specific combinations expressing heterosis due to non-additive gene action) can be multiplied vegetatively and taken for adaptability test and finally released to farmers Such specific hybrid combinations should be maintained and multiplied by asexual method.

5) The following pyrethrum breeding procedure is suggested:

a) Select good performing parents, test for their cross compatibility and cross the compatible ones using pair-wise planting them in isolated plots.

b) Develop the hybrid seedlings in the nursery and expansion plots.

c) The expanded hybrid seedlings should be split and planted per row or plot.

d) Performance of plants per plot or line should be evaluated and superior hybrids selected (hybrids that perform better than their parents did).

e) Superior hybrids can be taken for adaptability tests and released to farmers and or incorporated in the program as parents. Some superior hybrids that cross and produce inferior hybrids (specific combinations due to non-additive gene effects) can be multiplied vegetatively, taken for adaptability tests and released to farmers too.

6) Further studies on compatibility should be carried out to either determine whether under altered environmental conditions like temperature and humidity can break the self-incompatibility. This would enable the development of purelines that essential in hybridization and crop improvement.

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APPENDIX 1

A worked example of GCA, SCA and reciprocal effects on pyrthrins yields at Kabete.

Appendix 1A: Pyrethrins yields in kg/ha for six pyrethrum parents, their F_1 hybrids and reciprocals at Kabete.

Parents		Sb/66/107	P4	Ks/74/122	Ks/71/6	L/64/4331	Ks/70/64	Total
Sb/66/107	R_1	6.17	12.48	13.75	12.11	18.85	10.27	73.63
	R_2	5.87	14.96	15.97	15	33.18	10.1	95.08
	R_3	9.04	13.42	14.331	11.48	25.41	10.62	84.301
Total		**21.08**	**40.86**	**44.051**	**38.59**	**77.44**	**30.99**	**253.011**
P4	R_1	12.22	8.71	19.94	8.16	13.99	16.36	79.38
	R_2	7.29	9.44	16.94	10.35	11.5	16.38	71.9
	R_3	11.23	10	19.53	13.1	8.52	12.09	74.47
Total		**30.74**	**28.15**	**56.41**	**31.61**	**34.01**	**44.83**	**225.75**
Ks/74/122	R_1	15.64	14.49	5.86	11.35	8.57	14.98	70.89
	R_2	11.02	13.72	6.26	16.16	17.11	14.13	78.4
	R_3	15.49	15.01	8.94	12.64	16.38	15.14	83.6
Total		**42.15**	**43.22**	**21.06**	**40.15**	**42.06**	**44.25**	**232.89**
Ks/71/6	R_1	15.44	12.14	15.15	12.41	8.86	13.73	77.73
	R_2	10.63	17.85	13.66	8.29	5.37	10.07	65.87
	R_3	13.3	16.93	15.37	6.56	8.36	10.71	71.23
Total		**39.37**	**46.92**	**44.18**	**27.26**	**22.59**	**34.51**	**214.83**
L/64/4331	R_1	9.46	10.72	22.55	8.66	7.77	17.84	77
	R_2	15.52	9.58	14.89	8.84	8.72	17.88	75.43
	R_3	9.49	4.337	19.41	8.71	11.22	14.87	68.037
Total		**34.47**	**24.637**	**56.85**	**26.21**	**27.71**	**50.59**	**220.467**
Ks/70/64	R_1	10.69	18.52	13.96	11.06	16.42	10.85	81.5
	R_2	13.65	13.46	13.27	9.62	18.63	10.12	78.75
	R_3	15.13	17.47	18.58	8.64	12.49	11.56	83.87
Total		**39.47**	**49.45**	**45.81**	**29.32**	**47.54**	**32.53**	**244.12**

R_1, R_2 and R_3 = plot replications, replicate1, 2 and 3 respectively.

Total sum =1391.08. Total sum of squares =19975.377

Step I: Testing for genotypic difference.

Appendix 1B:Mean squares for pyrethrins yields among six pyrethrum parental clones at Kabete.

Source	Df	Sum of squares	Mean squares	F
Treatments	35	1576.29	45.04	6.556**
Replicates	2	0.529	0.265	0.00386
Error	70	480.94	6.87	
Total	107	2057.75		

Since calculated F value is larger than table F value at both 0.01and 0.05 probability levels, it implies that there were significant differences between the genotypes. Then we can proceed for further analysis

Step II: Combining ability analysis.

Appendix 1A above is rearranged as shown below (Appendix 1C).

Appendix 1C: Average pyrethrins yields of six pyrethrum parental clones, F_1 hybrids and their reciprocals

Parents.	Sb/66/107	P4	Ks/74/122	Ks/71/6	L/64/4331	Ks/70/64	Total (Yi.)
Sb/66/107	**7.03**	13.62	14.68	12.86	25 81	10 33	84 33
P4	10.25	**9.38**	18.8	10.54	11.34	14.94	75.25
Ks/74/122	14 05	14.41	**7.02**	13.38	14 02	14.75	77 63
Ks/71/6	13.12	15.64	14.73	**9.09**	7.53	11.5	71.61
L/64/4331	11.49	8 22	18 95	8.74	**9.24**	16 86	73 5
Ks/70/64	13.16	16.48	15 27	9.77	15 85	**10.84**	81.37
Total (Y.j)	69.1	77.75	89.45	64 38	83 79	79 22	463 69

Calculations for GCA, SCA, and Reciprocal sum of squares and mean of squares were then calculated as shown below.

1) Sum of squares due to GCA = ½n ((Yi. +Yj.)2 - 2/n²Y².. = 1/2x6[(84.33 +69.10)² +

(75.25 +77.75)² +. + (81.37 +79.22)²] - (463.69)² = 45.76

Therefore Mean of squares = 45.76/(6-1) = 45.76/5 = 9.152

2) Sum of squares due to SCA = ½ (Yij (Yij +Yji) - ½n ((Y.j +Yi.)2 - 1/n²Y²

=1/2[7.03(7.03 + 7.03) + 13.64(13.62 +10.25) + ...+ 16.86(16.86 + 15.85) +10.84(10.84

+10.84) - 1/2x6[(84.33 + 69.1)² + ...+ (81.37 + 79.22)²]+ 1/6x6(463.69)² =322.59

Therefore Mean of squares = 322.59/6(6-1)/2 =322.59/15 = 21.506

3) Sum of squares due to reciprocal = ½ ((Yij +Yji)²

=1/2[(13.62 -10.25)² + (14.68 - 14.05)² +.... + (16.86 -15.85)²] =157.08

Therefore mean squares = 157.08/6(6-1)/2 =157.08/15 =10.472

Step III: Testing significance of GCA, SCA and Reciprocal effects.

Mean square of Error = 6.87; divided by number of replications =6.87/3=2.29

Test of GCA effects: $F_{(5,70)}$ = GCA mean squares/2.29 = 9.152/2.29 = 3.997**

Test of SCA effects: $F_{(15,70)}$ = SCA mean squares/2.29 =21.506/2.29 =9.391**

Test of Reciprocal effects; F (15,70) = Reciprocal mean squares/2.29 =10472/2.29

=4.573**

GCA, SCA and Reciprocal effects are shown in Appendix 1D below.

Appendix 1D: GCA, SCA and Reciprocal mean squares for pyrethrins yields at Kabete six pyrethrum parental clones

Source	Degrees of freedom	Pyrethrin yields
GCA	5	9.2**
SCA	15	21.5**
Reciprocal	15	10.5**
Error	70	2.3
GCA: SCA ratio		0.14

*, ** Significant at probability 0.05 and 0.1

Calculations were done with reference to Appendix 1C above.

1). GCA effects = $1/2n(Yi. + Y.j) - 1/n^2Y.$

=1/2x6(84.33 +69.10) - 1/6x6(463.69) =-0.094 for parent Sb/66/107

Similarly all other values for each parent were calculated using the same method.

2). SCA effects =$1/2 (Yij+Yji) - 1/2n(Yi. + Y.i + Yj. + Yij) + 1/n^2Y..$

=1/2(13.62 + 10.25) - 1/6x6(84.33 + 77.75 + 75.25 + 69.10) + 1/36(463.69) =-0.721 for cross Sb/66/107 x P4.

SCA effects for other crosses were similarly calculated.

3). Reciprocal effects =1/2(Yij + Yji).

=1/2(13.62 - 10.25) = 1.69 for the reciprocal of Sb/66/107 x P4.

Reciprocal effects for other crosses were similarly calculated.

Appendix 1E: GCA, SCA and Reciprocal effects for six pyrethrum mother clones, F_1 and their reciprocals at Kabete for pyrethrins yield.

Parent	Sb/66/107	P4	Ks/74/122	Ks/71/6	L/64/4331	Ks/70/64
Sb/66/107	**-0.09**	-0.72	0.54	1.75	5.64**	-1.54
P4	1.69*	**-0.13**	2.81*	0.887	-3.20	2.46*
Ks/74/122	0.32	2.20**	**1.04****	1.69	2.33*	0.58
Ks/71/6	-0.13	-2.55	-0.68	**-1.55**	-3.43	-1.20
L/64/4331	7.16**	1.56*	-2.47	-0.61	**0.23**	2.75*
Ks/70/64	-1.42	-0.77	-0.26	0.88*	0.51	**0.50***

GCA effects are on the diagonal with SCA effects on the upper side and Reciprocal effects on the lower side.

*, ** Significant at 0.05 and 0.1 probability levels